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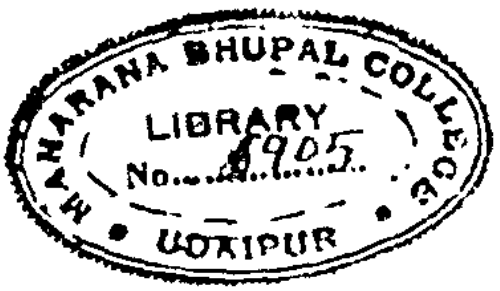

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COLOUR IN RELATION TO CHEMICAL CONSTITUTION OF DYES DERIVED FROM HETERO-CYCLIC NITROGEN CONTAINING DIBASIC ACIDS

BY

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In the arrangement of groups given by Dutt (J. C. S., 1927, *4*, 99) in order of increasing absorptive power, C : N group has been shown to be more absorptive than C : C group. Table I shows a comparison of the absorption spectra of a few of the homocyclic as well as the corresponding hetero-cyclic-nitrogen containing compounds.

TABLE I

Name	Absorption Spectra
Benzene	... 2490
Pyridine	... 2530
Toluene	... 2500
Picoline	... 2580
m-Xylene	... 2530
1 : 3 Lutidene ...	... 2770
Mesitylene	... 2570
Collidene	... 2830
Naphthalene ...	... 2760
Quinoline	... 2930
Anthracene	... 2950
Acridine	... 3300

A study of the above table shows that whenever there is a *nitrogen atom in a ring system within a molecule* the compound is invariably more coloured than the corresponding homocyclic compound containing carbon in place of nitrogen. Hence it was thought that a study of the dyes derived from heterocyclic dibasic acids together with the dyes obtained from corresponding carbo-cyclic acids would be of great interest from the point of colour in relation to chemical constitution. The dyes obtained from the *following four groups of acids have been chosen for* the study : (1) Quinolinic, Cinchomeronic and Phthalic acids. (2) Phenylpyridinedicarboxylic acid and diphenic acid. (3) Quinoline 1 : 2 : 3 tricarboxylic acid and naphthalic acid. (4) Imidazoledicarboxylic acid and triazoledicarboxylic acid. It has been found that dyes derived from Quinolinic (Ghosh, J. C. S., 1919, *115*, 1102) and Cinchomeronic (Tewari, J. C. S., 1929, *135*, 1642) anhydrides are slightly more absorptive than the corresponding dye-stuffs derived from phthalic anhydride. Though, of course, the brilliance

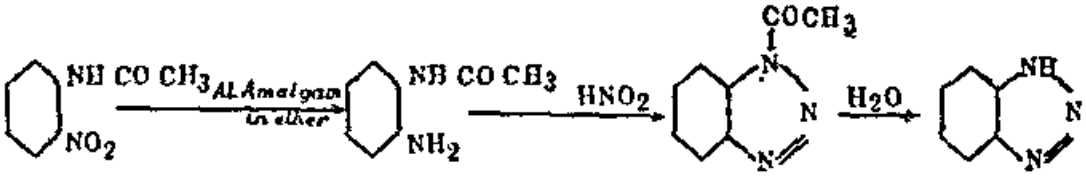
and covering power of the latter are undoubtedly greater. This will be apparent by having a look at Table No. III given on page 6. Similarly, it has been found that dyes derived from *s*. phenylpyridinedicarboxylic acid (Tewari and Dutt, Jour. Ind. Chem. Soc., 1926, *3*, 161) are more coloured than the corresponding dyes obtained from diphenic acid (Dutt, J. C. S., 1923, *123*, 225) and also dyes derived from quinoline 1 : 2 : 3 tricarboxylic acid (Tewari and Dutt, Jour. Ind. Chem. Soc., 1928, *5*, 59) are more coloured than the corresponding dyes derived from naphthalic acid (Terrisse. Annalen, 1885, *227*, 133) [*vide* Table No. IV]. Several dye-stuffs have also been prepared from triazoledicarboxylic acid and compared with the corresponding dyes derived from imidazoledicarboxylic acid (Tewari and Dutt, Jour. Ind. Chem. Soc., 1927, *4*, 26). Triazoledicarboxylic acid is a substitution product of imidazoledicarboxylic acid in which one of the carbon atoms of the ring has been replaced by a nitrogen atom. Triazoledicarboxylic acid was prepared as follows :

Preparation of orthonitroacetanilide.—This compound was prepared according to directions given in J. B. Cohen's *Practical Organic Chemistry*, pages 171-72.

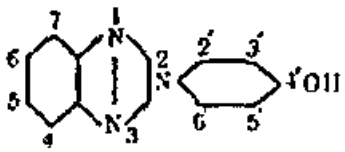
Reduction of o-nitroacetanilide.—Aluminium amalgam was added to solution of o-nitroacetanilide in moist ether until the yellow colour of the solution was discharged. Owing to its insolubility in ether acetyl-o-phenylenediamine separated out from the solution along with aluminium hydroxide, from which it was extracted by means of boiling benzene. It crystallises from benzene in small lustrous plates (M. P. 132°).

On addition of the calculated amount of sodium nitrite to a solution of this compound in hydrochloric acid an immediate precipitate of acetyl—1 : 2 : 3—benztriazole was produced, which was crystallised from alcohol in compact needles (M.P. 51°). Acetyl—1 : 2 : 3—benztriazole was

hydrolysed with extreme ease to give 1 : 2 : 3 benztriazole. (M. P. 98°).



1 : 2 : 3 benztriazole was oxidised with boiling alkaline potassium permanganate. The solution after filtering off the precipitated manganese dioxide was made neutral by the addition of sulphuric acid. It was then concentrated to a small volume when some of the potassium sulphate got crystallised out which was filtered off. The solution was then acidified with hydrochloric acid. The triazoledicarboxylic acid got precipitated. The yield obtained by this method was very poor. So the following method given by Chakarvarti and Dutt (Jour. Ind. Chem. Soc., 1928, 5, 557) was utilised for the preparation of the acid which gave a good yield. 2—(4′—hydroxyphenyl)—1 : 2 : 3 benztriazole



was prepared by reducing O-nitrobenzeneazophenol by alcoholic ammonium sulphide. It was obtained in colourless silky needles by crystallisation from alcohol. (M. P. 231°)

Oxidation of 2—(4′—hydroxyphenyl) 1 : 2 : 3 benztriazole.—Five grams of the above substance were dissolved in the requisite quantity of dilute sodium hydroxide and treated with a 5 per cent solution of potassium permanganate in the cold until the latter was no longer decolourised. The precipitated manganese dioxide was filtered off and the

filtrate was evaporated to a small volume after neutralisation with dilute sulphuric acid. The liquid was then rendered strongly acidic with dilute sulphuric acid when a crystalline precipitate was obtained, which was triazoledicarboxylic acid. It melted with vigorous decomposition at 200°. It was soluble in water and acetic acid, completely insoluble in acetone, ether, alcohol, ligroin and benzene. In comparing the two series of dye-stuffs, thus obtained, it is found that dyes derived from triazoledicarboxylic acid are invariably more absorptive than the corresponding dyes derived from imidazoledicarboxylic acid as the Table No. V on page 7 will show.

The following compounds have been condensed with triazoledicarboxylic acid and the corresponding dye-stuffs obtained : phenol, resorcinol, phloroglucinol, metadiethylamidophenol, and metaphenylenediamine. The preparation and purification of phenol, resorcinol, and phloroglucinol compounds were done just in the same way as in the case of imidazoledicarboxylic acid (Tewari and Dutt, Jour. Ind. Chem. Soc., 1927, *4*, 201). The condensation, isolation and purification of metadiethylamidophenol and metaphenylenediamine compounds were done exactly in the same way as in the case of the corresponding compounds of cinchomeronic acid (Tewari, J. C. S., 1929, *135*, 1642). The only difference was in the preparation of the diamine compound, which was done over an oil bath at temperature between 180°—200°, until the melt was dark-red in colour and became quite hard and brittle on cooling.

The properties of these are summarised in the form of a table (Table No. II).

TABLE II

Name	Appearance.	M. P.	Colour in Alkali.	Colour in Acid.	Colour of Fluorescence.	Analysis (Theoretical value in brackets).
Phenol-triazomaleein	Orange-yellow	Does not melt up to 275°	Pink-red	...	...	N=13·7% (13·59%)
Resorcinol-triazomaleein	Orange-red crystals	273°—275°	Orange-red	.	Bright-green.	N=13·4% (13·0%)
Phloroglucinol triazomaleein	Brown crystals	Does not melt up to 275°	Dark-red	..	...	N=12·0% (11·8%)
Metadiethylamidophenol-triazomaleein	Reddish-violet crystals	192°–194°	..	Bright-pink colour	Brown.	N=18·85% (18·1%)
Metaphenylenediamine-triazomaleein	Brown crystals	Does not melt up to 275°	...	Red	Green in alcohol	N=26·6% (26·2%)

TABLE III

Compound obtained from—, and	Phthalic Acid.	Quinolinic acid	Cinchomeronic Acid.
Phenol ...	5540	5560	5520
Resorcinol ..	4940	4950	4950
Phloroglucinol ...	4980	4990	4980
Metadiethylamidophenol ...	5540	5540	5540

TABLE IV

Compound derived from—, and	Diphenic acid.	β-phenylpyridinedicarboxylic acid.	Naphthalic acid.	Quinoline 1:2:3 tricarboxylic acid.
Resorcinol ..	4170	4650	4430	4900
Phloroglucinol ...	4220	4710	4490	4930
Metadiethylamidophenol ...	4290	5070	5140	5340

TABLE V

Compound derived from—, and	Imidazoledicarboxylic acid.	Triazoledicarboxylic acid.
Phenol	5510	5530
Resorcinol	4960	4970
Phloroglucinol ...	4970	4980
Metadiethylamidophenol	Could not be obtained in pure state.	5560

The figures in Tables Nos. I, III, IV, V, denote absorption maxima in approximate wavelengths.

My thanks are due to Dr. S. Dutt for the interest which he took in this work.

PHOTONITRIFICATION IN SOIL

BY

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In previous publications[1] from these laboratories a new view on the process of nitrification in soil has been advanced and supported by experiments. It has been shown that the process of nitrification in soil is mainly due to the oxidation of ammonium salts to nitrite by air in presence of sunlight. In other words, according to our view, nitrification in the soil especially in the tropics, is more photochemical in nature than bacterial.

In this paper, we are submitting the results of our experiments on nitrification in soil[2] effected by sunlight. The experiments were carried on as follows:

Soil was collected from the grass land from a particular spot in the laboratory compound digging a hole nine inches deep. The soil was crushed, passed through a sieve with one-millimeter bore, and was air-dried at 30°. Two thousand grams of this soil were mixed with 20 gms. of the ammonium salts and 750 c.c. water. The whole mixture was kept in both earthen and large glass jars having a capacity 4000 c.c. and exposed to sunlight. Water was daily added in order to make up for the water lost by evaporation. The vessels were weighed every week to test for the constancy in weight. The soil was well stirred every morning before exposure. Blank experiments were also carried on in the dark by covering the glass jars with a thick layer of black japan enamel and the earthen pots were covered with a tin lid blackened by black japan. For the experiments in the dark, these vessels were also placed

[1] J. Ind. Chem. Soc., 9, 1932.

[2] Soil Science, 31, 379, 1931.

in the sun to have the same temperature as those exposed in the sun. 50 gms. of the soil were taken out after the exposure was completed and the ammonia was estimated by adding potassium hydroxide to the soil and distilling the liberated ammonia, which was absorbed by a standard solution of sulphuric acid. At the end of the experiment, this sulphuric acid was titrated against the standard solution of caustic potash and thus the amount of ammonia absorbed by sulphuric acid was found. Then another 50 gms. of the soil were taken and treated with Devarda's alloy for estimating the ammonium salt and the nitrites and nitrates.

The following results were obtained with soil and ammonium salts exposed in earthen vessels for 160 hours.

TABLE 1

No.	Condition	Amount of salt added	Unchanged amount of salt	Amount of oxidised salt	Percentage oxidised
1	Sunlight ..	20 gms ammonium chloride	13 gms. ammonium chloride	6·7 gms. ammonium chloride	33·5
2	Dark ..	20 gms. ammonium chloride	18·7 gms. ammonium chloride	1·3 gms. ammonium chloride	6·5
3	Sunlight .	20 gms ammonium chloride to heated soil	15·5 gms. ammonium chloride	4·4 gms. ammonium chloride	22·0
4	Sunlight ...	20 gms. ammonium sulphate	18·75 gms. ammonium sulphate	1·2 gms. ammonium sulphate	6·0
5	Dark ..	20 gms. ammonium sulphate	20 gms. ammonium sulphate	nil	nil
6	Sunlight ...	20 gms. ammonium phosphate	16·6 gms ammonium phosphate	3·3 gms. ammonium phosphate	16·5
7	Dark ..	20 gms. ammonium phosphate	20 gms. ammonium phosphate	nil	nil

These experiments carried on in earthen pots were not very satisfactory because the pots being porous a good deal of the ammonium salts percolated and came out on the outer surface of the pots.

The following results were obtained in glass vessels after 160 hours' exposure to sunlight with loose covers :

TABLE II

No.	Condition	Amount of salt added	Unchanged amount of salt	Amount of oxidised salt	Percentage oxidised.
1	Sunlight ..	20 gms ammonium chloride	18·64 gms. ammonium chloride	1·33 gms. ammonium chloride	6·65
2	Sunlight ...	20 gms. ammonium phosphate	17·54 gms. ammonium phosphate	2·44 gms. ammonium phosphate	12·2
3	Dark ...	20 gms. ammonium phosphate	19·8 gms. ammonium phosphate	0·2 gm. ammonium phosphate	1·0
4	Sunlight .	20 gms. ammonium sulphate	19·6 gms. ammonium sulphate	0·3 gm. ammonium sulphate	1·5
5	Dark ..	20 gms. ammonium sulphate	20 gms. ammonium sulphate	nil	nil

As the percentage of oxidation was not high after 160 hours exposure, the vessels were again exposed to sunlight for a much longer period. The results tabulated on page 2 were obtained after a total exposure to sunlight for 700 hours in the months of May, June, and July.

Table III

No.	Condition	Amount of salt added	Unchanged amount of salt	Amount of oxidised salt	Percentage oxidised
1	Sunlight	20 gms. ammonium chloride	8·5 gms. ammonium chloride	15·25 gms. ammonium chloride	80·1
2	Dark	20 gms. ammonium chloride	17·25 gms ammonium chloride	0·85 gm. ammonium chloride	4·8
3	Sunlight	20 gms. ammonium sulphate	17·25 gms ammonium sulphate	2·62 gms. ammonium sulphate	13·2
4	Dark	20 gms. ammonium sulphate	20 gms. ammonium sulphate	nil	nil
5	Sunlight	20 gms. ammonium phosphate	2 gms. ammonium phosphate	16·5 gms. ammonium phosphate	89·0
6	Dark	20 gms. ammonium phosphate	19·5 gms. ammonium phosphate	0·25 gm. ammonium phosphate	1·4
7	Sunlight	10 gms. ammonium phosphate to heated soil	1·38 gms. ammonium phosphate	8·37 gms. ammonium phosphate	83·8

The foregoing results show conclusively that on exposing the ammonium salt solutions to light in contact with the soil, an appreciable amount of the salts are photochemically oxidised in air after an exposure for 160 hours but the oxidation is greatly increased after 700 hours exposure as shown in Table III. Ammonium phosphate undergoes oxidation to a greater extent than the other ammonium salts under similar conditions. The oxidation in the vessels kept in the dark under identical conditions is very small and in some cases negligible.

If nitrification is mainly bacterial as is generally believed to be, the ammonium salts in the vessels kept in the dark

should have undergone appreciable oxidation. But the results show that the dark oxidation is exceedingly small in comparison with the photochemical oxidation. Moreover, even with the sterilised (by heating for 48 hours at 150° C) soil ammonium phosphate undergoes considerable oxidation in presence of sunlight. The oxidation of ammonium phosphate in presence of sunlight is practically the same in the sterilised as well as in the unsterilised soils. It therefore appears that nitrification especially in tropical countries is *more a photochemical than a bacterial process.*

We have carried on similar experiments with urea. 10 gms. of urea were mixed with 1,000 gms. of the same soil and one glass jar was exposed to sunlight for 540 hours under the same conditions as in the case of ammonium salts. The following results have been obtained:

TABLE IV

Amount of urea added	Amount of unchanged ammonia	Amount of oxidised ammonia
10 gms.	4·7 gms.	0·7 gm.

Hence percentage of ammonification is 54 and the percentage of its subsequent oxidation to nitrites is 15.

From our experiments, it appears that ammonification is also markedly photochemical in nature. We have been able to convert several nitrogenous compounds into ammonia by exposing the solutions of these compounds to light. Hence it appears to us that in the soil the ammonification of nitrogenous compounds and the subsequent oxidation of ammonium salts to nitrites are markedly accelerated by light and the process of soil nitrification is more of photochemical than of bacterial origin.

Further experiments on this line on sterilised and unsterilised soils are in progress.

THE CONSTITUTION OF THE ACTIVE PRINCIPLE OF INDIAN RATI (SCARLET VARIETY)

BY

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The plant Rati or Ghungachi is mentioned by Susruta and the older Sanskrit writers, it must, therefore, have long been in use as a medicine among the Hindus; they describe two varieties, namely, red and white-seeded. Formerly, the root of the plant was considered to be a perfect substitute for liquorice, but experience has shown this to be erroneous. Sanskrit writers describe the root as emetic and useful in poisoning. Internally, the seeds are described as poisonous and useful in affections of the nervous system, and externally, in skin diseases, ulcers, affections of the hair, etc. The seeds reduced to a paste are recommended to be applied locally in sciatica, stiffness of the shoulder joint, paralysis, and other nervous diseases. In white leprosy, a paste composed of the seed and plumbago root is applied as a stimulant dressing. In alopecia a paste of the seed is recommended to be rubbed on the bare scalp. The seeds are used as a purgative, but in large doses are an acrid poison, giving rise to symptoms resembling those of cholera. The poisonous property is generally believed to be in the red covering of the seed. Taken internally by women, the seed disturbs the uterine functions and prevents conception. For the latter purpose, four to six seeds are swallowed every day, in two doses, for several days, after each menstruation.

The boiled seeds are said to possess powerful aphrodisiac properties. The seeds reduced to a paste are used for contusions and to reduce inflammation. Dr. Burton Brown (Punjab poisons) recorded a case in which forty seeds of Abrus (Rati), administered internally, caused purging and vomiting, with symptoms of collapse and suppression of urine; the patient recovered under the use of stimulants.

Rati or Jequirity as it is known in English is a plant of the natural order Leguminosæ. It is grown in India and other hot countries. It is a perennial twiner with numerous stems. Seeds from three to five are contained in pods which are from 1¼ to 1¾ in. long and ⅓ in. wide, flat, oblong, *truncate, with a sharp deflected beak, and finely silky.* The seeds are usually bright scarlet, with a black spot on the top, highly polished as if covered with red lac. The average weight of the seed is 1·75 grains. There are three varieties of the seed—scarlet, white and black, but the findings of the present paper are on the scarlet one. The seeds are used in India by the goldsmiths as weight.

Warden and Waddell ("Non-bacillar nature of Abrus poison," Calcutta, 1884) have given the name 'abrin' to the poisonous principle of Jequirity. They showed that abrin was closely allied to plant albumin but did not enter into any details as to whether it consisted of one or more proteids. Martin (Proc. Roy. Soc., 1887, 331—34), after removing the red cuticle of the seeds, proved the presence of two proteids—a globulin and an albumose—in the kernels. He classed the globulin to the group of vegetable paraglobulins and the albumose to Kühne and Chittenden's deutero-albumose. The albumose responded to the biuret reaction and was found identical with the α-phytalbumose of papaw-juice (Abs., 1886, 642). In a later paper Martin (Brit. Med. J., *2*, 1889, 184—87) established that the poisonous nature of the seeds is due to the two proteids only. Dr. Warden (Pharmacographia Indica, 1890, Vol. I,

p. 442) claims to have succeeded in isolating an acid from the seeds, which he named as 'abric acid' and represented it by the formula C_{21} H_{24} N_3 O_4. He also obtained a small quantity of pungent volatile oil. But he gives no details as to how abric acid was separated.

Abrin (The Chemistry of Plant Products, by Haas and Hill, 1928, Vol. I, p. 371) has been suspected to have the same properties as ricin which occurs in Ricinus. Their toxic characters have been attributed partly to the presence of ptomaine bodies and largely to bacterial toxins, a class of substance related to albumoses.

The above represents the work that has hitherto been done on the seeds of Rati. A systematic analysis of the seeds was, therefore, undertaken with a view to study the exact chemical nature of the poisonous constituents.

The powdered yellow kernels of the seeds on extraction with petroleum ether were found to yield 5·5 per cent of a non-drying yellow oil which had a strong pungent smell. The oil-free kernels on extraction with alcohol and evaporation of major portion of the solvent yielded a thick brown liquid which on cooling slowly deposited colourless needles. This substance contained nitrogen and on recrystallisation from boiling water was obtained as snow-white slender needles melting at 295°C. and having a molecular formula C_{12} H_{14} O_2 N_2. This substance has been named as 'abrine' by the present author. Warden (loc. cit.) must have got this substance in an impure form which gave him a wrong analytical data and made him suspect the compound to be acidic in nature.

Abrine is insoluble in all organic solvents excepting alcohol in which it is a little soluble. Neutral ferric chloride, lead acetate or sub-acetate has no effect on the substance. Phosphomolybdic acid produces a white precipitate which soon changes to yellowish green and finally to grey colour. Eardman's and Frohed's reagents give yellow coloration

with abrine. Gold chloride solution produces a yellowish turbidity which darkens and in about a minute becomes violet, which, however, throws down a blue-black precipitate on addition of concentrated hydrochloric acid. Platinic chloride seems to have no effect on the substance in the cold, but on heating and allowing it to cool a brown gelatinous precipitate settles at the bottom. Abrine is non-respondent to the rest of the alkaloidal reagents. It dissolves readily in hydrochloric acid and on complete evaporation of the solvent long silky needles are obtained which quickly dissolve in water. This must, therefore, be the hydrochloride of abrine. The hydrochloride gives all the reactions of the free base as described above, excepting that it has no reaction with gold and platinic chloride solutions. The hydrochloride, however, gives a white precipitate with phosphotungstic acid which turns brown in about five minutes. Abrine hydrochloride dissolves readily in water and very soon gets decomposed with the precipitation of abrine. In dilute nitric acid abrine dissolves and on spontaneous evaporation of water needle-shaped crystals of abrine nitrate are formed. This substance is, however, quite stable in water. Abrine forms a mono-picrate both in acetic acid and alcohol solution. It also forms a dibromo compound in cold alcoholic solution of bromine. From the bromo-derivative it is evident that abrine contains one double-bond linkage between two carbon atoms. From the colour reactions and salt formations it can be said that abrine is semi-alkaloidal in character. It is tasteless when pure and therefore may be classed in the group of non-bitter alkaloids.

The thick mother liquor left after the separation of abrine was diluted and clarified with lead acetate solution. The filtrate gave an yellow precipitate with basic lead acetate solution. The second lead salt on decomposition with sulphurated hydrogen in aqueous suspension and on complete evaporation of water gave an amorphous yellow

powder melting at 105°C. and having a molecular formula $C_{13}H_{14}O_7$. This does not reduce Fehling's solution and Tollen's reagent, but both are readily reduced if the compound is previously hydrolysed by warming with hydrochloric acid. It does not produce any coloration or precipitate with the usual alkaloid reagents. The substance has an astringent and mild bitter taste and produces a dark coloration with neutral ferric chloride solution which turns red on dilution. This substance has been named as 'Abralin' by the present author. The physiological examinations of abrine and abralin are in progress and will be published afterwards.

EXPERIMENTAL

The scarlet variety of Rati seeds was obtained from the local market. The red seed-coat was removed by coarsely crushing the seed in a grinding machine. The outer coating constituted 30 per cent of the entire seed. The yellow kernels were very hard and were crushed to powder by means of a powerful crushing machine.

50 gms. of the powdered kernel were freed from oil by petroleum ether in the cold and the oil-free powder on cold aqueous extraction gave a cloudy precipitate with ethyl alcohol showing the presence of enzymes. The powder on completely burning left 2·5 per cent of a white residue (ash) which on qualitative analysis was found to contain iron, calcium, aluminium, silicon, magnesium, phosphate and sulphate.

For complete analysis 1·5 kilograms of the crushed kernels were exhaustively extracted in a round bottom extraction flask with 5 litres petroleum ether (B. P. 35—60°C.), till a portion of the extract did not give any oily residue on evaporation of the solvent. From the petroleum ether extract 85 gms. of a yellowish brown non-drying oil was

obtained which had a pungent odour. The oil-free powder was successively extracted with rectified spirit. The first few extracts were yellow in colour and after that colourless extracts were obtained which deposited white needles in small quantities on evaporation of the solvent. The substance seemed to be very little soluble in alcohol and therefore the extraction was done about twenty times in order to recover the crystalline product completely from the powder. The powder became almost colourless after the extractions. The total alcoholic extract was concentrated under reduced pressure when a brown syrup having white crystalline suspension was obtained. The liquid, which had a disagreeable smell, was allowed to stand for about a week when the quantity of the crystalline product increased and settled at the bottom. It was filtered at the pump and the colourless residue was washed with water. The dried crude product, which weighed 13 gms., turned brown at 210°C. and completely melted at 247°C. This substance was moderately soluble in hot water from which snow-white needles were obtained melting at 295°C. This product contained nitrogen and was abrine.

The mother liquor after the separation of abrine was diluted with water and on addition of lead acetate solution a thick yellow flocculent precipitate was obtained. The precipitate, which had a strong disagreeable odour was washed free of lead and decomposed with H_2S. The yellow filtrate on complete evaporation of water gave a brown sticky substance from which no chemically pure substance could be isolated.

The filtrate of the above lead salt was yellow in colour and gave a bright yellow bulky precipitate with lead subacetate solution. The purified lead salt was decomposed by H_2S in alcoholic suspension. The yellow filtrate was concentrated under reduced pressure and on complete evaporation of the solvent an yellow deposit was obtained. It was non-crystalline in structure and melted at 105°C., after

remaining in vacuum desiccator for two days. This substance was 'abralin'.

The oil on purification with Fuller's earth and animal charcoal became lighter in colour. It did not contain nitrogen and sulphur and was tasteless. In a decimeter tube the oil rotated the plane of polarisation by +0·36°, thereby giving a dextro rotation of $[\alpha]_D^{35} = +0·39$. This small rotation must be due to the presence of sterols in the oil. The oil burnt with an absolutely non-luminous flame and gave the following constants :

Moisture	0·78%
Specific gravity at 25°C	0·9139
Refractive index at 25°C	1·4662
Acid Value	2·44
Saponification Value	191·7
Hehner Value	88·00
Acetyl Value	Nil
Iodine Value	95·1
Unsaponifiable matter	1·08

Abrine:—$C_{12}H_{14}O_2N_2$.—Abrine dissolved in concentrated nitric acid with orange-red colour which became yellow on dilution. In strong sulphuric acid it dissolved with yellow colour. When very slowly crystallised from water about 1 cm. long star-shaped needles were obtained. [Found: C, 65·78; H, 6·46; N, 13·15; M. W. (cryoscopic in phenol), 221.]

$C_{12}H_{14}O_2N_2$ requires C, 66·08; H, 6·42; N, 12·84; M. W. 218.

Abralin: $C_{13}H_{14}O_7$.—In concentrated sulphuric acid abralin dissolved with a yellow colour which slowly darkened and finally became deep red. On dilution the colour was discharged and an yellow flocculent precipitate separated, which was the aglucone of the glucoside. In strong nitric acid abralin dissolved with red colour, which became orange-yellow on dilution. It was optically active having a laevo

rotation of $[\alpha]_D^{35} = -27{\cdot}37$ in aqueous solution [Found: C, 54·98: H, 5·29; M.W. (cryoscopic in phenol), 286, (decomposition of the lead salt), 281. $C_{13}H_{14}O_7$ requires C, 55·32; H, 4·96; M. W., 282].

Abrine picrate : $C_{12}H_{14}O_2N_2 . C_6H_3O_7N_3$.—1 gm. of picric acid was dissolved in 30 c.c. alcohol and ·5 gm. of abrine was added. On slight warming, abrine dissolved and the colour of the solution slowly darkened and finally became orange-red. It was then heated to boil and allowed to stand over night. Next morning orange-yellow crystalline plates in clusters were formed. The mother liquor was decanted off and the crystals were washed free of picric acid with dilute alcohol. The picrate weighed 0·9 gm. and melted at 194°C. with decomposition. It was quite stable in presence of water in which it was very little soluble, forming faint yellow solution. (Found N, 16·06%. 15·92%; $C_{18}H_{17}O_9N_5$ requires N, 15·66%).

The picrate was also prepared in glacial acetic acid from which two different types of crystals were obtained—(1) orange-red needles in form of stars, and (2) very closely packed yellow soft needles. They were separated by mechanical means. Both melted at 194°C. with decomposition and the nitrogen content was 15·96 per cent and 15·98 per cent respectively. The orange-red variety changed colour and became perfectly yellow at 120°C. Thus all the three varieties were mono-picric acid salt of abrine having only different crystalline modifications.

Dibromo abrine : $C_{12}H_{14}O_2N_2Br_2$.—·5 gm. of abrine was put in a dry flask and alcoholic solution of bromine was added in the cold. The colour of bromine was discharged and abrine dissolved forming a light pink solution. Excess of bromine solution was added and was left for spontaneous evaporation at room temperature. After few days light yellow soft plates settled at the bottom and the mother liquor remained brown. Addition of water did

not separate the bromo derivative. Some acetone was next added when the solid deposit became colourless and the brown mother liquor was decanted off. It was thus freed from bromine by two more washings with acetone. The product on drying was obtained as whitish micro-crystalline powder. It slowly started turning dark from 220°C. and melted between 241-42°C., with decomposition. (Found Br, 42·7%; $C_{12}H_{14}O_2N_2Br_2$ requires Br, 42·3%.)

The author wishes to express his indebtedness to Dr. S. Dutt for the kind interest he has taken in the work. His thanks are also due to the Kanta Prasad Research Trust of the Allahabad University for a scholarship which enabled him to take part in the investigation.

CHEMICAL EXAMINATION OF SOME INDIAN MEDICINAL PLANTS

BY

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I. TINOSPORA CORDIFOLIA (MIERS)

Tinosporia Cordifolia, known as Gurach in Hindustani is a plant of the natural order Menispermaceæ. It is a well-known medicinal plant of long use in Hindu medicine. A glabrous, succulant, climbing shrub after reaching a great height and sending down long thread-like aerial roots. The bark is grey and creamy white. The branches bear smooth heart-shaped leaves and bunches of red berries. When dry, they shrink very much. The taste is very bitter, the odour not in any way peculiar.

As regards its medicinal properties, it is considered to be cold and dry by the Mohammedans. The fresh plant is said to be more efficient than the dry. It is taken with milk in rheumatism, acidity of the urine and dyspepsia. Tinospora Cordifolia attracted the notice of Europeans in India and has been formerly spoken of by them as a tonic, antiperiodic and diuretic. It is now an official in the Pharmacopœia of India and is introduced in Europe as a specific medicine. The medicinal property of the plant is said to be due to the presence of berberine. It is a remedial medicinal agent in the chronic diarrhœa and some forms of chronic dysentery. It was prescribed by ancient Hindu physicians in gonorrhœa with advantage. It is also regarded by natives in certain parts of India as a specific for the bites of poisonous insects and venomous snakes.

Flückiger in 1884 found the presence of berberine in small quantity in the stems by chloroform extraction of the calcified drug. The extracted mass was then boiled up with water and the filtrate gave a precipitate with tannic acid. The decomposition of the tannic acid salt gave a bitter principle which was ultimately proved to be a glucoside by hydrolysis with dilute sulphuric acid. Unfortunately, Flückiger could not crystallise the glucoside.

The above represents the work that has hitherto been done on this plant. The present work was, therefore, undertaken to throw some light on the constitution of the bitter principle.

The presence of the alkaloid was definitely established by the colour reactions of the usual alkaloidal reagents with the acidified solution of the alcoholic extract of the plant. But the quantity of the precipitate and the intensity of colours indicated the presence of the alkaloid in very small quantity. All attempts to isolate the alkaloid by different solvents proved unsuccessful. Alcohol extracted sufficient quantity of chlorophyll, sugar and traces of resins and waxes. The dilute hydrochloric acid extraction of the drug on neutralization with alkali precipitated all inorganic traces.

Aqueous extract of the plant gave precipitates with lead acetate and sub-acetate. But the decomposition of the precipitated lead salts with H_2S and on complete evaporation of the filtrate gave sticky brown masses containing sufficient quantity of reducing sugars. Chloroform, however, extracted two solid products in small quantities which were tasteless and non-alkaloidal in character.

Experimental

About 500 gms. of the dried and powdered stem of Tinospora Cordifolia were repeatedly and exhaustively extracted with distilled water. The extract completely

evaporated to dryness on water-bath. A brownish black-coloured hygroscopic powder was obtained which had a bad sugary odour and a sharp bitter taste. The yield was about 90 gms., *i.e.*, 18 per cent of the dried plant.

About 50 gms. of the dried and the powdered plant were extracted with cold dilute 1 per cent HCl for two days. The liquid was filtered and normal sodium carbonate solution was added in order to precipitate the alkaloid. A white flocculent precipitate was obtained which was proved to be purely inorganic and on analysis was found to contain calcium, sodium, nitrate, and traces of chloride.

10 gms. of the water extract were mixed up with 2·5 gms. of slaked lime, moistened with water and dried in a desiccator for three days. This was then extracted with various solvents,—petroleum ether, chloroform, ether and alcohol, but none did give any alkaloid although each fraction answered all the reactions of alkaloids.

5 gms. of the water extract were taken and dissolved in hot water. The solution was then clarified with lead acetate. The lead salt was separated and the filtrate after removing the excess of lead was evaporated to dryness. A dilute HCl solution of this sticky mass gave all the coloured reactions of the alkaloids, but it could not be crystallised from any organic solvents. The lead salt was also decomposed by H_2S and the filtrate evaporated to dryness also yielded sticky syrupy mass which could not be got as solid even after keeping it for several days in vacuum *desiccator.*

About 5 gms. of the water extract were refluxed for three hours with chloroform and alcohol separately. The corresponding extracts on evaporation yielded brown sticky products but the definite alkaloid could not be isolated.

The above attempts were made to isolate the alkaloid in free state but unfortunately no satisfactory results were obtained. But this is definitely ascertained, that there is

present in minute traces some substance alkaloidal in nature which cannot be isolated in the free state by any of the standard methods.

A few grams of the water extract were treated with ether and petroleum ether respectively, but on evaporation of the solvents no definite extract was obtained. 70 gms. of the solid water extract were refluxed several times with chloroform and the fractions collected. The extract was greenish yellow in colour. Chloroform was evaporated off when a solid residue of a whitish yellow colour weighing about 4 gms. was obtained. The whole product was then treated with hot alcohol several times which dissolved some portion. The residue was again dissolved in chloroform and on purification with animal charcoal, solid white waxy plates were obtained. The alcohol soluble solid was also similarly treated. The two compounds weighed only about 0·5 to 0·8 gm.

(*a*) *Alcohol soluble solid.*—White cylindrical prisms, (M.P. 76—78°C.) contains no nitrogen or sulphur. Soluble in alcohol and other organic solvents but insoluble in water; does not answer to any of the alkaloidal reagents. Reduces Fehling's solution on hydrolysis by dilute HCl; appears to be of glucosidal nature.

(*b*) *Chloroform soluble solid.*—White flakes, waxy in nature. Insoluble in alcohol, crystalline tendency to hexagonal shape. Shrinks at about 150°C. but melts completely at 173—74°C. Contains no nitrogen, does not reduce Fehling's solution even on hydrolysis. On combustion the results were (C=54·01%, H=7·02%, O=38·97%). Only one combustion could be done as the quantity of the substance was very small.

The remaining residue was then exhaustively extracted with alcohol. The alcoholic extract was concentrated and dried over water-bath. The extract was of a reddish-brown colour, extremely hygroscopic and tasting exceedingly bitter. It contained a good amount of free sugars. Attempts were

made to remove the sugar by acetone. All the ordinary organic solvents were tried but no pure solid could be recovered. However, a solid stuff was obtained after keeping it in a vacuum dessicator, which weighed over 13 gms., *i.e.*, 17 per cent. But this too was extremely hygroscopic and could not be crystallised. An acetyl derivative of the substance was prepared but that too could not be got in a crystalline form. Both the alcoholic extract and the acetyl derivative reduced Fehling's solution showing the presence of free sugars. One combustion was done which gave (C=57·8 %, H= 5·31 %).

2. SOLANUM XANTHOCARPUM

This plant which is known as Bhatkataya in Hindustani is of importance in Hindu medicine since long. The plant is very wild in India. Root is at least biennial, stem none, leaves frequently in pairs, oblong, armed on both the sides with long, strong, straight spines, bright blue flowers, calyx armed with straight spines, berries spherical, size of a large gooseberry, very smooth, when ripe yellowish green in colour.

The root is much esteemed as an expectorant and is used in cough, asthma, catarrhal fever, and pain in the chest. Kantakari, as it is known in Sanskrit, is used in medicine in various forms. The roots beaten up and mixed with wine are given to check vomiting. The juice of the berry is useful in sorethroat. It is also a good diuretic. According to Dr. Wilson (Calcutta, Medi. Phys., Trans., 2, 406) the stems, flowers and fruits are bitter and carminative, and are prescribed in those forms of the burning of the feet which are attended with vesicular watery erruptions. In Bengal, the plant is much used as a diuretic and in dropsy. In the Punjab hills, the juice of the plant is administered with black pepper in rheumatism. A decoction of the plant is used

in gonorrhœa. It is also thought to promote conception in female.

Regarding its chemical constituents, the fruits were found to give alkaloidal reactions corresponding to solanine. The dried leaves gave 20·7 per cent ash, containing a trace of an alkaloid and an astringent organic acid giving a green precipitate with ferric salts (Pharmacopœia Indica, 2, 553).

The above represents the work that has hitherto been done on the plant. The present work was undertaken with a view to systematically analyse it.

The dilute HCl extract answered to all the colour reactions of an alkaloid with the usual alkaloidal reagents. Alcoholic extract gave a beautiful crystalline solid which was suspected to be the alkaloid or the active principle and therefore, a good amount of the plant was analysed. This product was afterwards proved to be potassium nitrate.

Experimental

About 100 gms. of the dried plant was taken and well chopped into small pieces and made into as fine powder as was possible by mechanical beating. This was then filled up in big Soxhlet apparatus and extracted by different solvents in the following order: (1) petroleum ether, (2) chloroform, (3) ethyl acetate, (4) alcohol.

Petroleum ether extract.—(1·6 per cent) dark yellowish brown oily mass smelling of chlorophyll. The dilute HCl solution of the extract was tested for the presence of alkaloid but the results were negative.

Chloroform extract.—(1·4 per cent) soft, greenish yellow waxy extract admixed with a little oily material. Properties similar to the above.

Ethyl acetate extract.—(0·8 per cent) pale greenish waxy material. The dilute HCl extract gave a few tests for the presence of an alkaloid which could not be definitely

confirmed, nor anything pure could be isolated from the entire extract.

Alcoholic extract.—(12 per cent) pale greenish sticky solid, reduced Fehling's solutions very rapidly. Small portion was dissolved in dilute HCl and the solution answered to all the tests of alkaloids with the usual alkaloidal reagents. On dissolving the entire product in alcohol and after allowing it to stand for some time, beautiful white silky needles were separated which were filtered and recrystallised. The total yield of this crystalline product was 1·5 per cent. Very soluble in water. Does not melt at all. The combustion results showed only traces of carbon but contained nitrogen. It was then finally identified to be potassium nitrate. The alcoholic extract did not contain anything other than potassium nitrate and chlorophyll.

Attempts were made to prepare the lead salt in water extract of the plant and to obtain some solid product from lead salt by the decomposition of it by H_2S, but the lead salt on decomposition with H_2S and subsequent evaporation of the liquor gave brown sticky syrupy mass which could not be crystallised.

Examination of the ash.—A small portion of the dried and powdered plant was burnt in crucibles. The total ash amounted to 10·5 to 10·8 per cent. The soluble ash was about 8 per cent. The soluble portion was tested for inorganic radicals which showed the presence of magnesium, calcium, potassium, traces of iron, nitrate, sulphate and traces of chloride. The insoluble portion was mainly silica and some alumina.

Estimation of sugars.—About 50 gms. of the plant were boiled with distilled water exhaustively. The extract was clarified with lead acetate solution and the filtrate and washings were made to 1000 c.c. On titration with Pavy's solution, it was found to contain 1·6 per cent. of total reducing sugars on hydrolysis and 0·3 per cent without hydrolysis.

Enzymes—About 100 gms. of the plant (finely powdered) were taken in a flask to which some distilled water was added and kept for two to three days. After filtration alcohol was added, which precipitated a brownish coloured solid mass amounting to about 0·5 per cent. The plant, therefore, shows the presence of some enzyme even when it is perfectly dried.

The entire chemical examination of the plant did not give anything else which is interesting. It appears, that potassium nitrate which is present to the extent of about 2 per cent in combination with the traces of alkaloid may be said to be the active principle, and the medicinal properties of the plant may be due to its presence. It is well known that potassium nitrate is an excellent diuretic.

3. FUMARIA OFFICINALIS

Fumaria officinalis (shaheterah : Pitpapra in Hindi) of the natural order Fumareiaceæ is a herb very common in India. The dry plant is generally much broken up; mixed with it are many globular, smooth indehiscent capsules, the size of a large pin's head; seed single, dark brown, crested, odour hardly any, taste bitter, slightly acrid and astringent.

Several species of this family have long been used medicinally on account of the diuretic and the alterative properties. It is known to remove heptic obstructions, aparient and expellent of the humors but more specially of atrabilis. It is also a laxative and is beneficial in dyspepsia depending upon torpidity of the intestines and in scrobulous skin affections.

As regards its chemical examination, it is supposed to contain fumaric acid and a base known as fumarine observed first by Peschier and more fully described by Hammon [J. Chem. Med.,(3) VIII, 705]. The plant contains 5 to 6 per cent of the base to which the plant appears to have its physiological properties. It is separated from its salts by

caustic alkalis or their carbonates in the form of curdy precipitates which may be obtained as crystalline by spontaneous evaporation of its hot alcoholic solution. According to Preuss, fumarine crystallises in irregular six-sided *monoclinic prisms*, *soluble in alcohol*, *chloroform*, sparingly soluble in water, insoluble in ether. Its composition has not yet been determined.

So much is the amount of work that has hitherto been done on this plant. The present author was tempted to put the entire plant under systematic chemical examination on account of the large amount of organic base (5 to 6%) present in it. After a number of preliminary experiments a systematic extraction of the entire plant was performed.

Experimental

About 10 gms. of the dried powdered plant were extracted by dilute HCl. The extract was dirty red in colour, which was decolorised by animal charcoal. To different portions of this HCl extract, solutions of sodium carbonate, caustic potash and ammonium hydroxide were added. The precipitates were then separated off by filtration. The precipitates were then again dissolved in dilute HCl. The original HCl extract, the filtrates from the three fractions and HCl solution of the precipitates were all put to various tests for alkaloids. The original HCl extract and the filtrates (after being made acidic) answered to the presence of an alkaloid. The HCl solution of the precipitates did not give any alkaloidal reactions.

These experiments go to show that the precipitates obtained by the addition of solutions of sodium carbonate, caustic potash and ammonia did not at all contain any alkaloid. The precipitates on extraction with alcohol were found to contain no organic product. They were finally identified to be purely inorganic and are, therefore, the hydroxides and carbonates of some metals.

A few grams of the dried plant were exhaustively extracted with distilled water. The lead salt was then precipitated by the addition of a solution of lead acetate. The lead salt on decomposition by H_2S and consequent evaporation of the solvent to dryness yielded a syrupy brown sticky substance which could not be crystallised. The mother liquor also after removing the excess of lead on evaporation to dryness yielded a sticky substance containing large amounts of free sugars.

From another fraction of the water extract, tannic acid salt was precipitated. The precipitate was then mixed with lead carbonate and then was extracted with alcohol. No alkaloid could be recovered on evaporation of the alcohol to dryness.

About 100 gms. of the powdered plant were exhaustively extracted with water. The mother liquor on evaporation to dryness yielded a brown solid amorphous powder which amounted to about 29 gms. It smelt strongly of sugars and reduced Fehling's solutions immediately. About 10 gms. of this solid were then extracted by the following solvents: (*a*) petroleum ether, (*b*) chloroform, (*c*) ethyl acetate, (*d*) alcohol. The first three solvents did not give anything other than little waxy material. Alcohol, however, extracted some brown stuff which contained sugars in majority. The dilute HCl solution of this alcoholic extract answered to all the alkaloidal reactions; but the isolation of the alkaloid in free state could not be achieved.

About 100 gms. of the powdered plant were systematically extracted in a big Soxhlet apparatus with the following solvents:

(1) chloroform, (2) ethyl acetate, (3) alcohol.

Chloroform extract.—(4 %) semi-solid, deep green wax. Smelling of chlorophyll. A dilute HCl solution of this showed the presence of some alkaloid in minute traces.

Ethyl acetate extract.—(1%) soft greenish waxy material properties similar to above.

Alcoholic extract.—(13%) deep dark brown solid; smelling of charred sugars. Reduces Fehling's solutions immediately. No coloration with ferric chloride, faintly acidic to litmus. The dilute HCl solution of the extract showed the presence of a small amount of alkaloid. The majority of the extract contained sugars and resins.

Estimation of Ash.—Contains 20·9 per cent total ash. The soluble portion amounts to 14 per cent. On analysis of the soluble portion for the inorganic radicals it showed the presence of *calcium, potassium, sulphate, magnesium, and* traces of chloride. The insoluble portion was mainly silica and alumina.

Estimation of sugars.—The water extract of the dried plant on clarification by lead acetate solution was titrated with Pavy's solution. The total sugars amount to (7·7 to 8 per cent).

It will be apparent from the above account that the 5 to 6 per cent of the base obtained by Hammon from the hydrochloric acid salt by caustic alkalies or the carbonates are nothing else than inorganic hydroxides and carbonates of the metals such as calcium, magnesium and aluminium. The curdy precipitates described by him do not contain any trace of organic matter since it did not give any stuff on extraction with alcohol or any other organic solvents.

The author expresses his best thanks to Dr. S. Dutt for his invaluable guidance throughout the present investigations and to the Kanta Prasad Research Trust for a scholarship which enabled him to take part in the present investigations.

SECTION II

PHYSICS

ON THE ORIGIN OF THE SPARK LINES IN THE X-RAY SPECTRA

BY

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Siegbahn and Stenström[1] discovered as early as 1916 that the K_α lines of light elements are accompanied on their short wave-length side by some faint lines, the origin of which could not be explained. Since then, a large number of investigations have been made to decide the nature of these lines. Siegbahn and Doljsek[2] showed in 1920 that there were five lines accompanying the K_α line of elements Na (11) to K (19). Later researches by Coster,[3] Hjalmar,[4] Siegbahn and Larsson,[5] Thoraeus,[6] Druyvesteyn,[7] Lindberg,[8] Richtmeyer,[9] have proved that the satellite phenomenon is quite general and that most of the strong lines in the X-ray spectra (K_{α_1}, K_{β_1}, L_{α_1}, L_{β_1}, L_{β_2}, L_{α_2}, M_α) exhibit it. The chief characteristics of these satellites are the following :

(1) They generally accompany a strong line towards the short-wave side although some satellites to the

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[1] Siegbahn and Stenström, *Phys. Zeit.*, *17*, 48, (1916).
[2] Siegbahn and Doljsek, *Zeit für Physik*, 10, 59, (1922).
[3] Coster, *Phil. Mag.*, *44*, 456, (1922).
[4] Hjalmar, *Phil. Mag.*, *41*, 625, (1921).
[5] Siegbahn and Larsson, *Ark für Mat Astr. och Fysik*, *Band* 18, *Nr.* 18 (1924).
[6] Thoraeus, *Phil. Mag.*, 2, 1107, (1926).
[7] Druyvesteyn, *Zeit für Physik*, 43, 707, (1927).
[8] Lindberg, *Dissertations, Upsala University.*
[9] Richtmeyer, *Phys. Rev.*, 34, 574, (1929).

long-wave side are also known, *e.g.*, L_{γ_1} line of some elements while K_{α_1} line of others have satellites to the long waveside.

(2) Coster[1] in 1922 and Bäcklin[2] in 1924 have shown that these lines require a higher excitation potential than the diagram line which they accompany.

(3) That the difference in frequency between the satellite and the parent line is of the same order of magnitude for the different elements and different series.

(4) These satellites are broad and diffuse and it is quite likely that a higher resolving power of the spectrograph will reveal a structure in them.

(5) There seems to be an upper limit for elements which show satellites in a particular series; for example, the K_{α_3} line has not been found beyond As. The satellites K_{α_3} and K_{α_4} have not been found beyond Cl. The satellites of the L-series show a similar behaviour. These facts are dealt with in a later part of the article.

Wentzel's Theory.

The first attempt to explain these lines was made by Wentzel[3] in 1921. He suggested that the satellites are due to multiply ionised state of the atom. When the atom is doubly ionised, the charge on the nucleus increases because of the change in the screening; and hence radiations of still shorter wavelength are emitted. The chances of an atom becoming multiply ionised are small and

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[1] Coster, *Phil. Mag.*, *44*, 456, (1922).

[2] Bäcklin, *Zeit für Physik*, 27, 30, (1924).

[3] Wentzel, *Ann der Physik*, 66, 437, (1921).

consequently the intensity of the satellite is small. Thus, according to Wentzel, the X-ray satellites stand in the same relation to diagram lines as spark lines stand to the arc lines in the optical spectra. Wentzel gave a scheme with the help of which he explained all the satellites of $K\alpha_1$ line. Druyvesteyn, some years later, explained other spark lines in the L-series in a similar manner.

TABLE 1

Line	Origin		Kind
	Initial State	Final State	
α_2	KL	L^2	Second
α_4	K^2	KL	Second
α'	K^2	K^2L	Third
α_3	KL^2	L^2	Third
α_5	K^2L	KL^2	Third

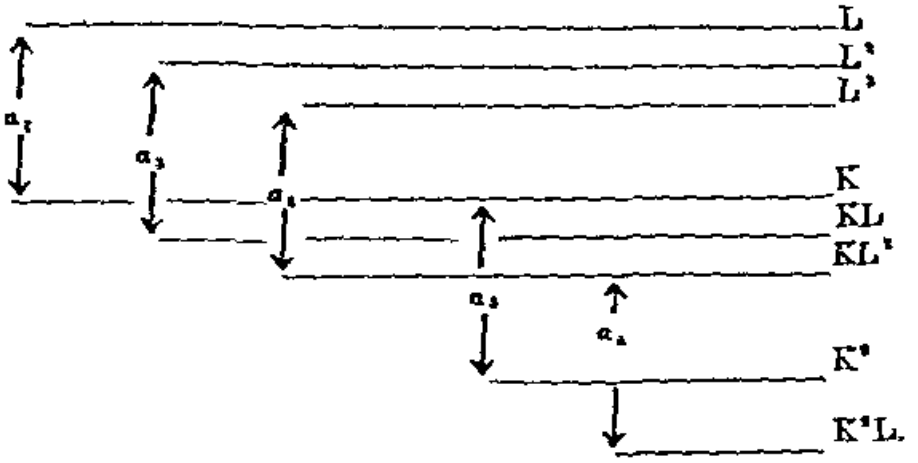
KL means that the K-shell as well as the L-shell is singly ionised.

KL^2 means that the K-shell is singly ionised and the L-shell is doubly ionised.

Second means double ionisation, and third means triple ionisation.

Wentzel supposed that each spark line results from one kind of multiply ionised state of the atom, and in order to explain the five lines accompanying the K line he takes into account two doubly ionised, and three triply ionised states of the atom. Thus $K\alpha_3$ results from the transition from a singly ionised K-shell and a singly ionised L-shell to a doubly ionised

L-shell. Hence its origin is denoted by $KL—L^2$. The figure below is an explanatory diagram of Wentzel's scheme.



The lines $K\alpha_1$ K_{α_2}, and K_{α_4} originate from those initial states of the atom in which the K-shell is singly ionised and hence $\alpha_3 - \alpha_1 = \alpha_3 - \alpha_2$.

Again, K_{α_3} and K_{α_5} originate from those initial states of the atom in which the K-shell is doubly ionised, and hence the atom behaves as though it has the next atomic number. The conclusion is

$$(\alpha_4 - \alpha_3)_z = (\alpha_3 - \alpha_1)_{z-1}$$

These two deductions are the chief arguments in support of Wentzel's theory. Wetterblad's[1] measurements made in 1927 support the above relations within experimental errors. Druyvesteyn,[2] arguing on similar grounds, proposed that the origin of the satellites of the K_β line can be ascribed to the transition KL←LM. Further, as

$$(KL) \approx K_z + L_{z+1}$$

and $$(LM) \approx L_z + M_{z+1}$$

he obtains $$(KL - LM)_z - (K-M)_z = (L_{z+1} - L_z) - (M_{z+1} - M_z)$$

or in other words

$$(\beta'' - \beta_1)_z = (L_{z+1} - L_z) - (M_{z+1} - M_z)$$

[1] Wetterblad, *Zeit für Physik*, 42, 611, (1927).
[2] Druyvesteyn, *Zeit für Physik*, 43, 707, (1927).

This curve when plotted does not tally exactly with the curve obtained experimentally. It is quite clear that Wentzel's theory may have some points in its favour, but it cannot explain the whole phenomenon in a satisfactory manner. At the time when Wentzel gave his theory, Pauli's principle was not known and complex spectra were not understood. We know now that the ionised states postulated by Wentzel will give rise to far greater number of lines than have been discovered. Moreover, the K-level cannot be triply ionised, for in the normal state of the atom there can be only two electrons in that shell. The chances of an atom being ionised triply are very small and so it cannot account for $K\alpha_1$ and $K\alpha_2$ lines which are fairly strong. Many workers have pointed out that Wentzel's suggestion in its original form is untenable. Several other suggestions have been put forth and in all these the main idea of Wentzel about multiple ionisation stands intact. Another important point conclusively established by Bäcklin[1] was that the excitation potential of these lines is much less than double the excitation potential for the diagram lines which is demanded by Wentzel's theory.

Before dealing with alternative theories, it is very important to discuss the fact as to how the atom comes to be in the multiply excited state and what the mechanism can be. Wentzel's original suggestion was that the multiple ionisation occurs when the atom is successively bombarded by two electrons. Rosseland[2] in 1923 dealt with this question from the theoretical point of view and came to the conclusion that the life in the excited states of the atom is very small and the probability of its encountering another cathode particle is too small to account for the spark lines.

Bäcklin[3] showed that the intensity of the spark lines increases linearly with the current instead of varying as the

[1] Bäcklin, *Zeit für Physik*, 27, 30, (1924).
[2] Rosseland, *Phil. Mag.*, 45, 65, (1923).
[3] Bäcklin, *Zeit. für Physik*, 27, 30, (1924).

square of the current. Hence on the theoretical as well as practical grounds, the successive encounter theory is untenable. Proceeding from Thomson's ionisation theory, Rosseland showed that the probability of two electrons being dislodged in a single encounter was of the same order of magnitude as would explain the observed intensity of the spark lines. Druyvesteyn[1] attacks the problem in the same manner but instead of supposing that two electrons are knocked out in a single encounter with the same cathode particle, he assumes that the two electrons are removed in successive encounters.

The arguments in favour of Rosseland's[2] view are:

The excitation potential must be considerably higher in the case of spark lines than in the case of diagram lines accompanying them. Bäcklin[3] in 1924, and Richtmeyer[4] and Jesse Du Mond in 1930 have proved it experimentally. But whether the excitation potentials of spark lines correspond to the sum of the excitation potentials of the levels excited is a question which is difficult to answer, for the experimental difficulties are enormous and have not been overcome.

The arguments against double ionisation in single collision are:

(1) From optical spectra there is no conclusive evidence that two electrons can be ejected in at one encounter. Goudsmit and Pauling[5] have deduced some general conclusions regarding the simultaneous transition of two electrons. They are identical with the rules holding in the optical spectra; it amounts to saying that of the two transitions, one should be allowed, the other should be forbidden. But numerical values for such transitions have not been obtained.

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[1] Druyvesteyn, *Zeit für Physik*, 43, 707, (1927).
[2] Rosseland, *Phil. Mag.*, 45, 65, (1923).
[3] Bäcklin, *Zeit für Physik*, 27, 20, (1925).
[4] Richtmeyer and Jesse Du Mond, *Phys. Rev.* 36, 1044, (1930).
[5] Goudsmit and Pauling, *Structure of Line Spectra*, p. 90.

From experiments on the production of optical spectra by Franck's method of cathode ray bombardment it is known in many cases that *multiply charged* ions are produced and they appear to be produced in single encounter, but this has not been clearly established.[1]

(2) This phenomenon should show itself in the absorption also where we expect a discontinuity at the place *corresponding to the energy required to* remove two electrons at the same time. Several observers have studied the absorption spectrum with this end in view. It is well known that when a beam of continuous X-rays is passed through matter the resulting spectrum shows absorption discontinuities at the limits of K, L, M, N ... series. They are due to the removal of an electron by the photon from the K, L, M, N, ... shells. A question now arises that if a photon is capable of removing two electrons simultaneously, say, one from *K-shell* and the other from *L-shell*, there should be an absorption discontinuity at

$$\nu = \nu_{K_i} + \nu_{L_i} \text{ approximately.}$$

Such an absorption discontinuity has not been clearly established, though from time to time several investigators announced the existence of the phenomenon. Coster and Van der Tuuk[2] tried it in argon but with negative results. Wentzel[3] reported that Siegbahn has observed the phenomenon but the latter never published anything about it, probably because he could not prove it conclusively. Richtmeyer[4] experimented with Sn, but did not

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[1] Bleakney, *Phys. Rev.*, 36, 1303, (1930).
[2] Coster and Van der Tuuk, *Zeit für Physik*, 37, 367, (1926)
[3] Wentzel, *Zeit für Physik*, 31, 445, (1925).
[4] Richtmeyer, *Phys. Rev.*, 27, 794, (1926).

come across the expected discontinuity in the absorption spectrum.

From a discussion of values of absorption coefficients for Cu over a wavelength region extending from K to half the value of K-region, Alexander[1] in 1927 showed that an absorption discontinuity occurs at approximately half the K-limit. This was subjected to adverse criticism by Richtmeyer.[2] At the present time it does not appear that the presence of such absorption discontinuity at the wave-length corresponding to KK, KL, LL, LM, etc., has been definitely established. But we may point out that the difficulty of observing such a phenomenon has not been properly appreciated. It is well known that the value of the discontinuity at the K-limit

$$\delta = \frac{n_K + \Sigma n_L}{\Sigma n_L}$$

where n_K is the number of photo-electrons released from K-shell and Σn_L is the number of electrons released from the L-shell. The discontinuity makes itself felt at the K-limit. Because as long as ν is less than ν_K, $n_K = 0$. Now the extent of the discontinuity will depend upon the value of n_K or on the number of photo-electrons released in the new process that has come into existence. If this is very feeble, the discontinuity may not be observed at all. For example, in the L_1-discontinuity, we have

$$\delta = \frac{n_{L_1} + n_{L_{2'}} + n_{L_{2''}}}{n_{L_{2'}} + n_{L_{2''}}} = 1{\cdot}20$$

In these cases the discontinuity may not be observed at all and actual experience tells us so. In the case which we have got in view, the value of the supposed absorption discontinuity at $\nu = 2\nu_K$, we shall have

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The value of s will depend on n_{KK}, *i.e.*, the number of processes in which two K-electrons are ejected simultaneously.

On *a priori* grounds, therefore, the probability is small, s will be approximately unity, and unless very sensitive apparatus is used it is not possible to establish the existence of such discontinuity to the satisfaction of everybody.

A very important experiment was done by Coster and Druyvesteyn[1] on the origin of spark lines. This was the well-known method of fluorescent excitation. It is known that when an element is irradiated by X-rays of sufficient high frequency, it emits its own characteristic frequency. The condition is that the frequency of the irradiating radiation should be higher than ν_K if we want to get the K-spectrum of the element. Coster and Druyvesteyn illuminated iron target by Cu K_α radiation and found that not only the diagram lines $K\alpha_1$, α_2, $K\beta$, are excited but the $K\alpha_3$ is also excited. If we assume that this is a spark line this experiment clearly proves that the incident $\nu_{K\alpha_1}$ of copper removes two electrons from K_1 and L_1 in one single stroke but alternative explanation is also possible as has been done by Coster and Druyvesteyn. It may be that the Cu K_α releases one K-electron from iron and imparts to it the remaining part of the energy; $\frac{1}{2}mv^2 = h\nu_{Cu K\alpha_1} - h\nu_{Fe K\alpha}$.

The electron as it passes out from the K_1- level knocks out another electron from the L- or M-level, and thus produces double ionisation. The process is thus analogous to that of internal conversion of gamma rays as worked by Ellis.[2] The intensity of the spark line should then vary with the frequency of the exciting radiations in a way regarding which guidance may be obtained from Ellis's theory of internal conversion. In many other ways the experiments of Coster and Druyvesteyn can be improved. For example,

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it can afford us valuable information regarding the minimum excitation potential required for the production of different spark lines.

For example, if K_{α_2} is produced as a result of double ionisation consisting in the removal of one electron from K. and another from L_1, the minimum value of the exciting frequency should be $\nu/R = 577{\cdot}4$, in the case of iron as shown below.

Fe	K_1- excitation level	$= 524{\cdot}0$
	L_1 " "	$= 53{\cdot}4$
	$K_1 + L_1$ excitation	$= 577{\cdot}4$

Hence K_{α_2} should be excited only when Fe is irradiated by Ni K_β whose ν/R value is equal to 608·7 but should not be excited when Fe is irradiated by Ni K_{α_1}, for ν/R in this case is 550.

From considerations to be given later, all the satellites may not have the same origin; K_{α_3}, K_{α_4} may be due to single ionisation in K_1- shell, and single ionisation in L_1- shell, but K_{α_5}, K_{α_6} may be due to double ionisation in the K- shell. This hypothesis can be tested by Coster and Druyvesteyn's method. If our theory be correct K_{α_5, α_6} can be produced in fluorescence when the irradiated radiations have double the ν_K/R of iron. It cannot be produced by the K_{α_1} radiations of elements from Copper up to Sr, but can be exeited by Strontium K_{β_1}.

ν_K/R of Fe	$=$	524
$2\nu/R$ of Fe	$=$	1048
$\nu_{K_{\beta_1}}/R$ of Sr	$=$	1166

Note.—For these Figures see Lindh's contribution in *die Handbuch der Experimental Physik*, XXIV—2, page 232.

There are certain evidences of an indirect nature regarding the simultaneous ejection of two electrons by a single photon in Robinson's[1] experiments on the magnetic spectrum of photo-electrons released by X-rays. The nature of

[1] *Robinson, Proc. Roy. Soc.*, A **128**, 29, (1930).

these experiments are well known. Radiations of some elements are allowed to fall upon a metallic plate. Under the action of these rays, photo-electrons are given out which are analysed by means of a magnetic field such that photo-electrons of the same velocity are focussed at one point of the photographic plate. By knowing the radius of curvature of the path of the electron from the geometry of the apparatus and also from the value of the magnetic field used we get the velocity of the electron. In this way, the energies of the photo-electrons can be studied. If we subtract the energy of the photoelectron so obtained from the energy of the radiation used we get the energy required to dislodge the electron from the level which contained it. This value must agree with the characteristic level values obtained from X-ray spectroscopic data.

But Robinson has obtained some faint lines which cannot be explained as being due to the release of a single electron from any known levels.

It cannot be proved from Robinson's experiments that two electrons are simultaneously ejected, one from L-shell and the other from M-shell. But against this hypothesis we may suppose, in a way similar to Coster and Druyvesteyn, that the quantum releases an electron from the L-shell and imparts to it the energy 1353. As it goes out, it knocks off another electron from L, or any other upper levels in the same way as in Ellis's internal conversion. There seems to be no criterion in Robinson's experimental results which can enable us to decide between the theory of simultaneous ejection of two electrons by a photon or a bombarding electron, or the theory of single ejection and internal conversion.

Alternative Theories of the Origin of Spark Lines.

(1) B. B. Ray's[1] theory based on theory of complex optical spectra.

[1] B B. Ray, *Phil. Mag.*, 8, 772 (1929)

(2) Langer's[1] modification on B. B. Ray's theory.
(3) Richtmeyer's[2] double jump theory.

B. B. Ray's Theory.

At the suggestion of Prof. M. N. Saha,[3] B. B. Ray first applied the principles of complex optical spectra to explain the origin of these lines, but all the points could not be cleared; and recently an alternative theory on the same lines has been proposed by Langer. In the following discussion we confine ourselves to the K spark lines.

According to B. B. ray, the mechanism of production of spark lines is as follows: The bombarding electron removes at one blow one electron from K_1, the other from L_1. The constitution of the inner incomplete shells is now 1s.2s giving the terms $^1S_0$, $^3S_1$. Then an electron jumps from L_2 to K. The electron constitution of incomplete shells is now $2s.2p^3$. These give us the spectroscopic terms ^1P, $^3P_{0,1,2}$. Hence we should obtain the following multiplet.

	^1P	$^3P_0$	$^3P_1$	$^3P_2$
$^1S_0$	a_1		a_4	
$^3S_1$	a''	a_3	a_5	

The arrangement of the lines in the form of a multiplet is due to B. B. Ray.

N.B.—Sometimes a sixth line close to a_5 but distinct from is and called by a' has been obtained.

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[1] Longer, *Phys. Rev.* **37**, 457 (1931).
[2] Richtmeyer, *Phil. Mag.*, **6**, 64 (1928)
[3] Saha and Ray, *Phys. Zeit.* **28**, 221, 1927.

The process may be described by the symbols now in use

$$1s^2.\ 2s.\ 2p^4 \longleftarrow 1s.\ 2s.\ 2p^5$$
$$^1P_1\ \ ^3P \longleftarrow {}^1S_1.\ {}^3S_1$$
$$\left(\ \underline{1s} \longleftarrow \underline{2p}\ \right)$$

While this theory explains a few features of the spark lines it does not explain why we get 5 lines in place of 6 spark lines which are expected. It may be mentioned that Dr. G. B. Deodhar,[1] working in Siegbahn's laboratory got a sixth line between a_5 and a_6 in silicon, and denoted it by a_7. But it is not clear why silicon alone should show this line, and not the other elements. Deodhar also showed that a_3 is a close doublet.

Langer proposes that the origin of the lines may be represented by the following symbol:

$$1s^1 2s 2p^3 \longleftarrow 1s 2s 2p^3$$
$$^1S.\ \ ^3S \longleftarrow {}^1P\ {}^3P.$$

This is merely B. B. Ray's theory repeated, with the addition that $^3P_{012}$ is regarded as a single term, the differences being too small for observation. According to Langer the intercombinations do not occur, hence this transition accounts only for two lines.

	$^1P_1$	$^1P_{012}$
$^1S_0$	a_1	—
$^3S_1$	—	a_4

The other lines are supposed to be due to the process:

$$1s^1\ 2s^2\ 2p^4 \longleftarrow 1s\ 2s^2\ 2p^3$$
$$^1P_1\ \ ^3P \longleftarrow {}^1S_0.\ {}^1D_2\ \ {}^3P$$

[1] Deodhar, *Proc. Roy. Soc.* A **131**, 633 (1931).

i.e., the bombarding electron knocks out one electron from K_1 and one from L_2, and then one electron jumps from the L_2 level to K, giving to the configuration $1s^2.\ 2s^2.\ 2p^6$. Again, we count the three terms of ^3P as one term, and suppose that the intercombinations are absent. We obtain

	^1P	^3P
$^1S_0$	×	
$^1D_2$	×	
^3P		×

There is a very important point which none of these theories have sought to explain. It is found that after sulphur, α_3 and α_6 lines can no longer be traced α_4 disappears after Ca rather it becomes merged in α_3 and ultimately α_3 is the only line to persist. This has been traced up to As. [*Vide*, however Coster and Druyvesteyn. Under ordinary circumstances, α_3 is the strongest line having approximately $\frac{1}{2}\frac{1}{5}$ of the intensity of α_1 while α_5 and α_6 are very faint.

Light on this point is thrown by the experiments of Bäcklin on the excitation potentials of the spark lines of Al. According to Wentzel's original suggestion, two electrons are to be ejected from the K_1-level for exciting the spark lines called α_4. The minimum excitation potential should therefore be at least double the excitation potential of the K-lines. For Al, this is nearly 1,500 volts, and Bäcklin found that α_4 appears at a much lower voltage than the minimum voltage calculated from Wentzel's theory,; in fact it appears when V is about 1,700 volts. He concludes that this result is distinctly against Wentzel's suggestion that both K-electrons have to be

ejected for exciting certain lines of spark spectrum. *On this point B. B. Ray's theory is helpful, as the excitation* potential of spark lines should exceed the excitation potential of K-lines only slightly, namely by V_L where

$V_L = \frac{h\nu_L}{e}$, and V_L for Al is only about 100 volts.

But a closer study of Bäcklin's paper shows that the α_5 and α_6 lines come out first as very faint lines, only when the excitation potential is 2,900, *i.e.*, about double the K-potential, In Deodhar's experiment as well, the lines α_5 and α_6, appear as very faint lines compared to α_3 and α_4.

These considerations and disappearance of α_5, α_6 after Sulphur led me to the hypothesis that the origin of the two sets of lines is probably different, α_3 and α_4 are due to the process

$$1s.^2\ 2s.\ 2p^3 \longleftarrow 1s.\ 2s.\ 2p^4$$
$$1s. \longleftarrow 2p$$
$$^1P_1,\ ^3P \longleftarrow {}^1S_0\ \ 3S.$$

but α_5 and α_6 are due to

$$1S^2\ 2S\ 2p^4 \longleftarrow 2(s)^2\ 2(p)^2$$
$$^1P_1,\ \ ^3P \longleftarrow {}^1S_0$$

i.e., in the second process the bombarding electron carries away both electrons in the K-shell, leaving the constitution as $2s^2 2p^4$. Then two electrons simultaneously jump one from $2p$ to $1s$, the other from $2s$ to $1s$. Thus one transition is allowed, the other is forbidden. We thus get the lines

	^1P	^3P
$^1S_0$	α_5	α_6

If this hypothesis be correct, the actual frequency of α_5 and α_6 should be in the region of double the frequency of the $K\alpha$-lines, or the wave-length should be half. In other

words the lines called α_5 and α_6 have been recorded in the second order. Their faintness and non-appearance for heavy elements are due to two causes: (1) As their excitation potential should be double the excitation potential of the K-lines, the potential used in the heavy element was probably not sufficient to excite these lines. Thus for Cu, the excitation potential for K-lines = 9,000 volts, and excitation potential for α_5 and α_6 should be 18,000 volts. If we want to obtain the lines sufficiently intense, with small exposure at least double this voltage ought to be used. In the experiments on spark lines, of elements after sulphur the voltage was probably kept low, hence the lines did not appear. (2) When we expose for the $K\alpha$ region, as has been usually done. $1s^2.2s.2p^5 \longleftarrow 1s.2s.2p^6$ lines provisionally called $(\alpha_3\alpha_4)$ are exposed for the first order, while the $\alpha_5\alpha_6$ lines have approximately half the wavelength of K-lines, hence they are exposed for the second order. Hence even with sufficient secondary voltage, they appear very faint.

This hypothesis regarding the origin of the several spark lines may or may not explain all facts but it suggests the existence of a new class of characteristic lines (to which the author was led also from another independent line of reasoning) which owe their origin to the simultaneous ejection of two electrons from any shell, and simultaneous jumping of two electrons from some outer shell to fill up these vacant positions. Such lines will have approximately double the frequency of the usual diagrammatic K-and L-lines, and their structure will be similar to complex spectra in optical spectroscopy. The existence of such double transition L-lines in Tungsten has already been demonstrated.

My most sincere thanks are due to Professor M. Saha, D.Sc., F.R.S., for suggesting me to write this article, and the active help and advice given in writing it.

TABLE II

Satellites of $K\alpha$-line

Atomic number	Element	α'		α_3		α_4		α_5		α_6		α'	
		$\lambda \times 10^7$	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$
11	Na	11835	76·99	11802	77·21	11781	77·35	...	77·77	...	77·98	11885	76·68
12	Mg.	9827	92·73	9799	93·00	9786	93·12	9730	93·66	9712	93·83	9867	92·34
13	Al	8286	109·99	8265	110·26	8253	110·42	8206	111·05	8189	111·28	8319	109·53
14	Si	7083	128·66	7064	129·00	7051	129·19	7014	129·92	7003	130·13	7109	128·18
15	P	...	...	6102	149·34	6093	149·51	...	...	...	...	6141·71	148·37
16	S.	5341	170·62	5329	171·00	5323	171·19	5263	173·15	5247	173·67	5360·9	169·98
17	Cl	4703	193·76	4688	194·38	4684	194·55					1718	193·14
19	K	3719	245·03	3711	245·56	3709	245·69					3733	244·07
20	Ca			3332	273·49	3330	273·66					3351	271·88
21	Sc			3006	303·15							3025	301·24
22	Ti			2727	334·17							2743	332·2
23	Va			2485	366·71							2498	364·15
24	Cr			2273	400·90							2285	398·83
25	Mn			2088	436·43							2097	434·49
26	Fe			1923	473·88							1932	471·60
27	Co			1777	512·8							1785	510·44
28	Ni			1648	552·96							1654	550·75
29	Cu			1531	595·20							1537	592·75
30	Zn			1429	637·70							1432	636·34
31													
32													
33	As												

TABLE III

Satellites of $K\beta$-lines

Atomic No.	Element	$K\beta_1$		$K\beta'$		$K\beta_2$		$K\beta_{2''}$		$K\beta'$
		λ	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$	λ	$\frac{\nu}{R}$	
19	K	2446·8	264·28	3443·0	264·67	3431·6	265·32	3400·7	267·96	
20	Ca	2083·4	295·51	3079·7	295·80	3069·1	296·92	3044·3	299·33	
21	Sc	2773·9	328·51	2767·5	329·27	2758·0	330·41	2742·5	332·28	
22	Ti	2509·0	363·20	2500·7	363·49	2493·7	364·42	2483·6	366·91	
23	V	2279·7	399·72	2272·6	400·98	2264·6	402·40	2257·7	403·61	
24	Cr	2080·3	437·98	·	...	2067·0	440·93	2061·7	442·0	
25	Mn	1905·9	478·057	·	..	1892·2	481·32	1888·8	482·18	
26	Fe	1752·7	519·6	...	...	1740·6	523·48	1737·1	524·6	

TABLE IV

Satellites of $K\beta$-lines (Beuthe)

Atomic No.	Name	β_η (Same as β'' of Druyvesteyn.)		β_η (Same as β')
		λ	$\frac{\nu}{R}$	
23	V	2256·8	403·8	
24	Cr	2061·1	442·13	
25	Mn	1890·4	482·06	
25	Mn	1885·8	483·23	
26	Fe	1737·7	524·41	
27	Co	1603·4	569·34	
30	Zn	1281·9	710·87	
31	Ga	1205·4	756·00	
		1195·9	762·18	
		1193·8	763·34	
32	Ge	1117·4	815·54	
33	As	1046·6	870·70	
34	Se	982·6	927·70	
35	Br	923·6	986·76	
37	Rb	820·4	1110·8	
38	Sr	775·3	1175·4	
39	Y	733·2	1242·9	

SECTION III

MATHEMATICS

NOTE ON BESSEL FUNCTIONS

BY

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In Article 1 of the present paper some expansions in terms of Bessel functions are obtained and in Article 2 certain integrals involving Bessel functions of zero order are evaluated.

1. Certain Expansions in terms of Bessel Functions. It is known that*

$$e^{\frac{1}{2}z\left(t-\frac{1}{t}\right)} = \sum_{-\infty}^{\infty} J_n(z)\, t^n \ldots \qquad (1.01)$$

Differentiate (1.01) with respect to t and put $t = e^{i\theta}$

$$\tfrac{1}{2}ze^{\frac{z}{2}\left(e^{\theta i}-e^{-\theta i}\right)}\left(1+e^{-2\theta i}\right) = \sum_{-\infty}^{\infty} nJ_n(z)\, e^{(n-1)\theta i}$$

Equating real and imaginary parts, we have,

$$\tfrac{1}{2}z\left[\cos(z\sin\theta) + \cos(z\sin\theta - 2\theta)\right]$$

$$= \sum_{1}^{\infty} n\, J_n(z)\, \left[\cos(n-1)\theta + (-)^{n-1}\cos(n+1)\theta\right] \ldots (1.02)$$

And

$$\tfrac{1}{2}z\left[\sin(z\sin\theta) + \sin(z\sin\theta - 2\theta)\right]$$

$$= \sum_{1}^{\infty} n\, J_n(z)\left[\sin(n-1)\theta - (-)^{n-1}\sin(n+1)\theta\right] \quad \ldots . (1.03)$$

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* Whittaker and Watson, *Modern Analysis*, Fourth Edition, p. 355.

Differentiate (1.02) twice with respect to θ and put $\theta=0$. We have,

$$z\,[z^2-2(z-1)]=\sum_1^\infty n[(n-1)^2+(-)^{n-1}(n+1)^2]J_n(z)\ldots(1.04)$$

Differentiate (1.03) with respect to θ and put $\theta=0$. We have

$$z\,(z-1)=\sum_1^\infty n\,[(n-1)-(-)^{n-1}(n+1)]J_n(z)\quad\ldots\qquad(1.05)$$

2. Evaluation of Certain Definite Integrals.

From the known result*

$$Y_0(x)=-2\int_0^\infty \cos(x\cosh u)\,du$$

we have

$$Y_0(ax)=-2\int_0^\infty \cos(ax\cosh u)\,du\ldots.\qquad(2.01)$$

Multiply both the sides of (2.01) by $\frac{1}{(1+a^2)^s}$ and integrate between the limits 0 and ∞. Then

$$\int_0^\infty \frac{Y_0(ax)}{(1+a^2)^s}\,da=-2\int_0^\infty \frac{da}{(1+a^2)^s}\int_0^\infty \cos(ax\cosh u)\,du$$

Changing the order, this becomes

$$\int_0^\infty \frac{Y_0(ax)}{(1+a^2)^s}\,da=-2\int_0^\infty du\int_0^\infty \frac{\cos(a\,x\cosh u)}{(1+a^2)^s}\,da$$

But $$\int_0^\infty \frac{\cos mx}{(1+x^2)^s}\,dx=\frac{\pi}{4}(1+m)e^{-m}$$

Therefore,

$$\int_0^\infty \frac{Y_0(ax)}{(1+a^2)^s}\,da=-\frac{\pi}{2}\int_0^\infty (1+x\cosh u)e^{-x\cosh u}\,du$$

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* *Quarterly Journal*. Vol. 42.

which by virtue of the formula

$$K_n(x) = \int_0^\infty \cosh n\phi \, e^{-x\cosh\phi}\, d\phi$$

becomes

$$\int_0^\infty \frac{Y_0(ax)}{(1+a^2)^{\frac{1}{2}}}\, da = -\frac{\pi}{2}\{K_0(x)+xK_1(x)\} \ldots \quad (2.02)$$

But it has been proved that*

$$-\pi K_0(x) = \int_0^\infty \frac{Y_0(ax)}{(1+a^2)^{\frac{1}{2}}}\, da$$

Therefore (2.02) gives

$$-\pi x K_1(x) = \int_0^\infty \frac{1-a^2}{(1+a^2)^2}\, y_0(ax)\, da \quad \ldots \quad (2.03)$$

Other results proved in the same way with help of the following integrals.

$$\int_0^\infty \frac{\cos mx}{(1+x^2)^3}\, dx = \frac{\pi e^{-m}}{16}(m^2+3m+3)$$

$$\int_0^\infty \frac{\cos mx}{(1+x^2)^4}\, dx = \frac{\pi}{96} e^{-m}\left[15+15m+6m^2+m^3\right]$$

are

$$\int_0^\infty \frac{Y_0(ax)}{(1+a^2)^3}\, da = -\frac{\pi}{8}\left[\frac{x^2}{2}K_2(x)+3xK_1(x)\right.$$

$$\left.+\left(3+\frac{x^2}{2}\right)K_0(x)\right] \ldots \quad (2.04)$$

$$-\pi x^2 K_2(x) = \int_0^\infty \frac{4(1-3a^2)-x^2(1+a^2)^2}{(1+a^2)^3} Y_0(ax)\, da \ldots (2.05)$$

---

* *Quarterly Journal*, Vol. 42.

$$\int_0^\infty \frac{F_0(ax)}{(1+x^2)^2}\,dx = -\frac{\pi}{48}\Big[3(5+x^2)K_0(x) + 3x\left(5+\frac{x^2}{4}\right)K_1(x) + 3x^2K_2(x) + \frac{x^3}{4}K_3(x)\Big]\ldots \quad (2.06)$$

2.1. *The integral* $\int_0^\infty \frac{aJ_0(ax)}{(1+a^2)^2}\,da$

It is known that

$$J_0(ax) = \frac{2}{\pi}\int_0^\infty \sin(ax\cosh u)\,du \ldots \quad (2.07)$$

Multiply both the sides of (2·07) by $\frac{a}{(1+a^2)^2}$ and integrate between the limits 0 and ∞.

We have,

$$\int_0^\infty \frac{aJ_0(ax)}{(1+a^2)^2}\,da = \frac{2}{\pi}\int_0^\infty \frac{a\,da}{(1+a^2)^2}\int_0^\infty \sin(ax\cosh u)\,du$$

$$= \frac{2}{\pi}\int_0^\infty du\int_0^\infty \frac{a\sin(ax\cosh u)}{(1+a^2)^2}\,da$$

on changing the order of integration.

But

$$\int_0^\infty \frac{x\sin mx}{(1+x^2)^2}\,dx = \frac{\pi}{4}\,m\,e^{-m}$$

Therefore

$$\int_0^\infty \frac{aJ_0(ax)}{(1+a^2)^2}\,da = \frac{x}{2}\int_0^\infty \cosh u\,e^{-x\cosh u}\,du$$

$$= \frac{x}{2}K_1(x) \quad \ldots \quad \ldots \quad (2.08)$$

In a similar manner, by the help of the integral

$$\int_0^\infty \frac{x\sin mx}{(1+x^2)^2} = \frac{\pi}{16}(m^2+m)\,e^{-m}$$

t can be shown that

$$\int_0^\infty \frac{\alpha J_0(\alpha x)}{(1+\alpha^2)^3}\, d\alpha = \frac{1}{16}\left[x^2 K_0(x) + 2x K_1(x) + x^2 K_2(x)\right] \quad (2.09)$$

2.2. Product Formulæ

Multiply both the sides of (2.08) by $J_0(x)$, then,

$$\frac{x}{2} K_1(x)\, J_0(x) = \int_0^\infty \frac{\alpha\, d\alpha}{(1+\alpha^2)^2} J_0(\alpha x)\, J_0(x) \quad \ldots (2.10)$$

By virtue of the known result

$$J_0(x)\, J_0(y) = \frac{1}{\pi}\int_0^\pi J_0\left\{\sqrt{(x^2 + y^2 - 2xy\cos\theta)}\right\} d\theta$$

we obtain from (2.10)

$$\frac{x}{2} K_1(x)\, J_0(x) = \int_0^\infty \frac{\alpha\, d\alpha}{(1+\alpha^2)^2} \int_0^\pi J_0\left\{x\sqrt{(1 + \alpha^2 - 2\alpha\cos\phi)}\right\} d\phi$$

By making the transformation

$$-1 + \alpha\cos\phi = \rho\cos\theta,\ \alpha\sin\phi = \rho\sin\theta$$

$\alpha\, d\alpha\, d\phi$ is changed into $\rho\, d\rho\, d\theta$ and

we have

$$\frac{x}{2} K_1(x)\, J_0(x) = \frac{1}{\pi}\int_0^\pi \int_0^\infty \frac{\rho\, d\rho\, d\theta\, J_0(x\rho)}{(2 + \rho^2 + 2\rho\cos\theta)^2}$$

But since

$$\int_0^\pi \frac{d\theta}{(a + b\cos\theta)^2} = \frac{a\pi}{(a^2 - b^2)^{3/2}}$$

therefore

$$\frac{x}{2} K_1(x)\, J_0(x) = \int_0^\infty \frac{\rho J_0(x\rho)\, d\rho\, (2 + \rho^2)}{(\rho^2 + 4)^{3/2}}$$

Putting $\rho^2 = 2 \sinh \phi$ we get

$$x\, K_1(x)\, J_0(x) = \tfrac{1}{2} \int_0^\infty \frac{1+\sinh\phi}{\cosh^2\phi}\; J_0\left(x\sqrt{2\sinh\phi}\right) d\phi \quad \ldots \quad (2.11)$$

Similarly we obtain from (2.09)

$$\tfrac{1}{8}\left[x^2 K_0(x) + 2x K_1(x) + x^2 K_2(x)\right] J_0(x) = \int_0^\infty \rho \left[\frac{2(2+\rho)^2 + 4\rho^2}{(\rho^2+4)^{5/2}}\right] J_0(x\rho)\, d\rho \quad \ldots \quad (2.12)$$

SECTION IV

BOTANY

A STUDY ON THE RESPIRATION OF FRUIT OF CARICA PAPAYA (*VERN. PAPITA*) IN RELATION TO CHANGES IN SUGAR CONTENT

BY

U. N. CHATTERJI, B.Sc. (Hons.), M.Sc.

INTRODUCTION

The study of fruit ripening from the biochemical point of view had long been neglected. But in the last few years much work has been done in this field especially with stored apples, so that all the possible aspects of the ripening and biochemical changes of this fruit have been thoroughly investigated.

Archbold, working on the various problems of the ripening of apples, came to the conclusion that their "growth as measured by dry weight proceeds at an increasing rate until the last month on the tree, when it is very slow." She found close correlation between "starch and acid accumulation," and, that the "soluble carbohydrate increase at first, remain constant while starch is being stored and finally increases rapidly." This progressive conversion of starch into sucrose and finally into simpler sugars during the ripening of apples has also been emphasised by other workers, *e.g.*, Evans, Bigelow, etc., working on the chemical composition of pears. Emmett found that reducing sugars accumulate because of the rate of oxidation being slower than that of inversion, and that the "development of soluble pectin is the chief factor concerned in the softening of the fruit."

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Blackman and Parija have investigated into the respiratory changes of stored apples and found the rate of respiration increases towards their senescent phase. The cause of this rise is, according to them, "the lowering of organization resistance" during senescence. A study of the variations in respiratory rate and changes in the sugar content during the ontogeny of the fruit of Carica papaya has been made in this paper.

MATERIAL AND METHOD

The fruits of Carica papaya were gathered from a local garden. The size of the full-grown fruits influenced the selection of the tree beyond anything else as very big fruits would be difficult to manage. Weight, size and position on the tree were taken to be the chief factors determining the age and different stages of the ontogeny of the fruits. The colour of the fruits when fully ripe was somewhat reddish yellow.

For measuring respiration the well-known air-commutator devised by Blackman, with Pettenkoffer tubes, was used. The whole apparatus consisted of four parts.

(1) A thermostat bath was maintained at a temperature of 34°C to 35°C. In it were kept the respiratory chambers which were wide-mouthed glass jars covered with black cloth. The mouth was fitted with a rubber-stopper carrying two glass tubes bent at right angles—one connected to air-commutator and the other to another bottle filled with strong potassium hydroxide solution—to absorb carbon dioxide from the ingoing air-current.

(2) The air-commutator is so well-known that it hardly needs any description. It is a device by which the respiratory current is automatically shifted on to the next Pettenkoffer tube after some fixed interval of time. This interval was three hours throughout this work.

(3) Then there were the Pettenkoffer tubes each filled with 25 c.c. of baryta water of known strength with about 40 c.c. of water to absorb the carbon dioxide from the respiratory current which bubbled through the solution.

(4) The Pettenkoffer tubes were connected to an aspirator which dropped at a uniform rate, thus drawing in a slow and constant current.

The content of each Pettenkoffer tube was poured out after the regular respiratory current had passed through it and titrated with standardised hydrochloric acid. The amount of carbon dioxide absorbed was calculated from the difference in the quantity of the acid used to neutralise this and 25 c.c. of baryta directly.

For sugar estimations a piece of the fruit was weighed out and then boiled in water to kill the enzymes. The piece was then crushed and the paste thoroughly mixed with the same water which was used to kill the enzymes. The whole mass was then filtered by means of a Buchner Funnel. Lead acetate was added to the filtrate for precipitating tannin. This was filtered and the extra amount of lead in the filtrate was got rid of by repeatedly passing hydrogen sulphide. The surplus amount of hydrogen sulphide was boiled off. The volume of the solution was measured and the amount of sugar was estimated by titrating against Pavy's solution. For the estimation of disaccharides a known volume of the leaf extract was previously boiled with about 1c.c. of concentrated hydrochloric acid and then neutralised with sodium bicarbonate. The difference between the results obtained with hydrolised and unhydrolised fruit-extracts multiplied by ·95 gave the amount of disaccharides.

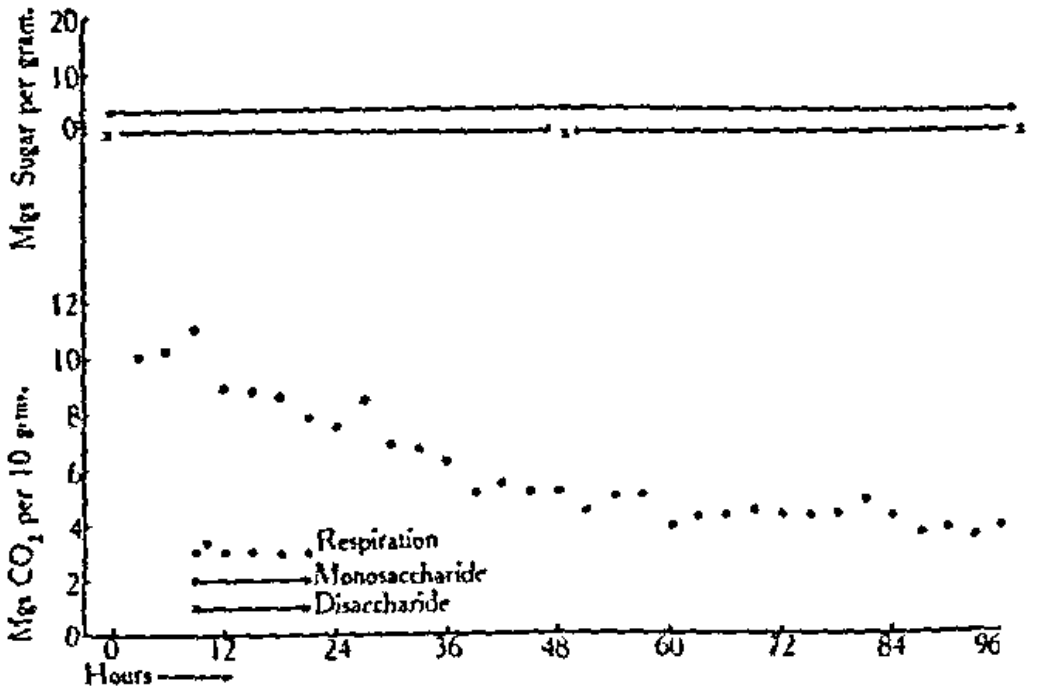
In all seven experiments were done and all were run for 96 hours. For starting an experiment three fruits of approximately the same size and weight were chosen—one was washed with potassium permanganate solution and kept in the respiratory chamber for measurement of CO_2 output,

a second was kept in reserve in another bottle in same bath and connected directly to an aspirator and the remaining one was treated for sugar estimation. The second sugar estimation was made from the reserved fruit after 48 hours, and the third from the one kept in the respiratory chamber after 96 hours, *i.e.*, after the termination of the experiment.

EXPERIMENTS AND INDIVIDUAL RECORDS

It will be advantageous to examine the individual records of each experiment before passing on to the general discussion of the results. For only a study of the experimental data will bring out the points to be discussed. In all seven experiments were done and the first seven graphs represent the result of each experiment. In the first experi-

Graph No. 1.

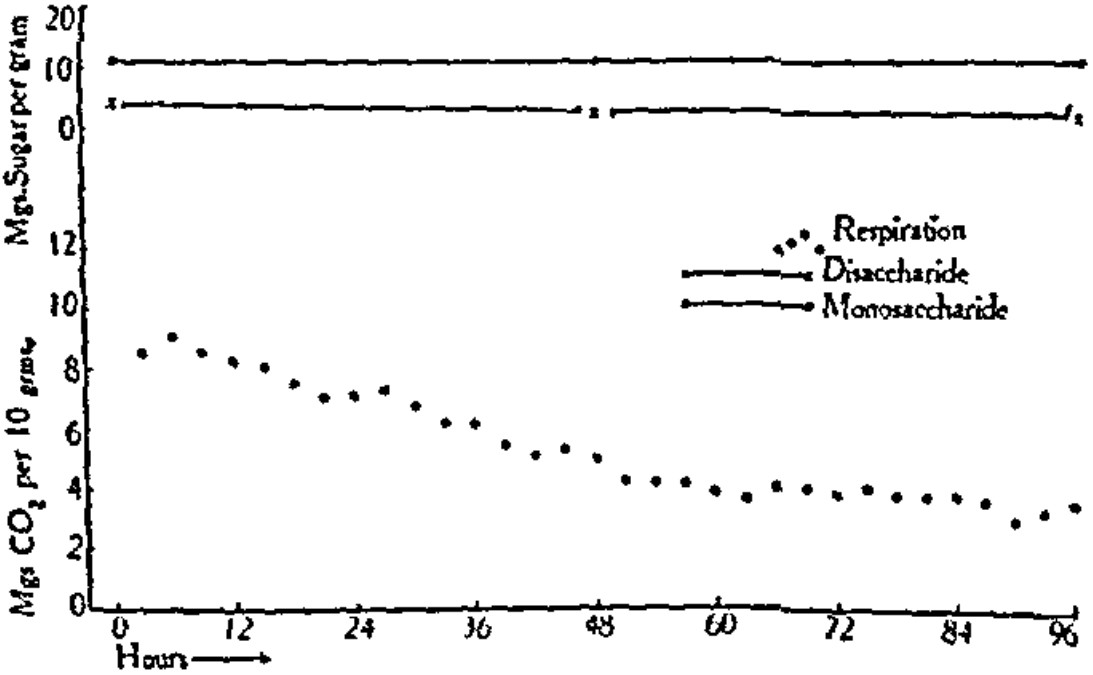


ment (Graph No. 1) the fruit was very small weighing only 11·9 gms. The CO_2 output begins at 10 mgs. and gradually

comes down to lower than 4 mgs. The sugar content--both monosaccharides and disaccharides—is very small and does not show any appreciable falling off.

The same condition of things obtains in the second graph, although here the initial rate of respiration is lower than in the previous case and the amounts of both disaccharides and monosaccharides have increased.

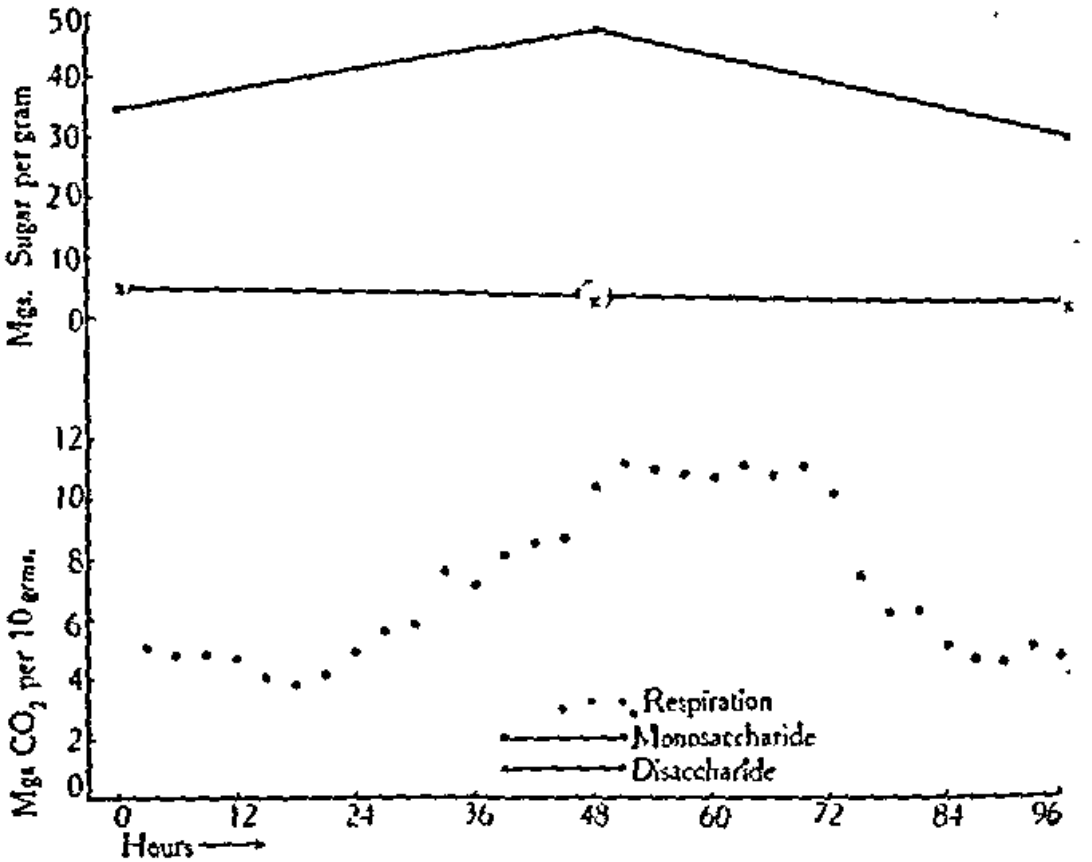
Graph No. 2.



In the third graph the monosaccharides content has gone considerably up; but not so the disaccharides which retain very much the same position they were in previously. Respiration begins at a lower rate from the very beginning, and goes on falling till after the sixtieth hour when it attains almost a constant rate. But contrary to the fall in CO_2 value that of sugar is not appreciable.

sixth hour after which descent comes about rather abruptly. The yellowish patch had considerably increased and had taken a deeper hue when the fruit was examined after the twenty-fourth hour. When seen after the forty-eighth hour

Graph No. 6.

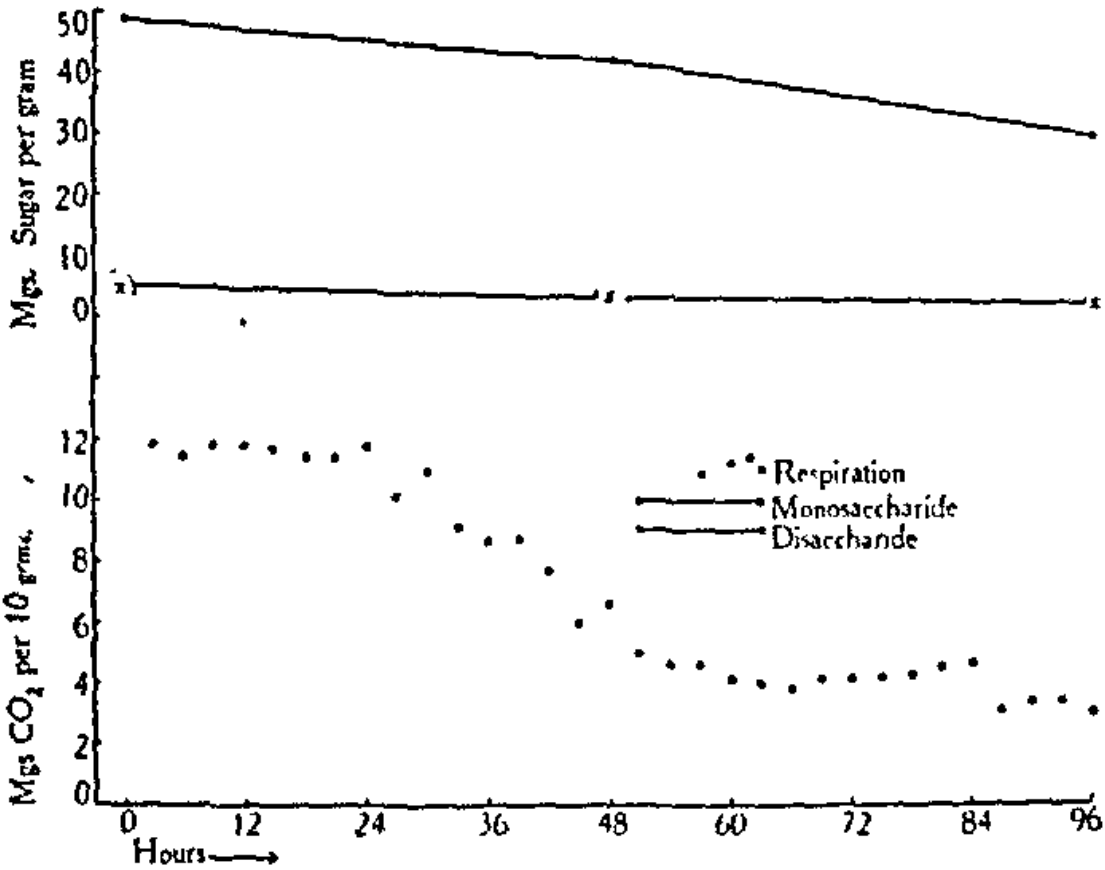


almost the whole fruit was reddish yellow and soft and velvety to touch, and remained so when it was taken out after the conclusion of the experiment.

In accordance with the humped nature of the respiratory curve, the monosaccharide content shows a corresponding rise and fall, but the amount of disaccharides remains constant. The rise in the respiratory rate causes a heavy drain on the simpler sugars which are oxidised and therefore more of the polysaccharides must be hydrolysed in order to keep pace with the accelerated oxidation process and this may be the cause of its sudden increase.

A quite ripe fruit was employed in the seventh experiment. It was quite reddish-yellow at the time it was put

Graph No. 7.



into the chamber. The CO_2 value begins at high level, remains almost constant for about twenty-seven hours, then falls and becomes rather parallel with horizontal at about the sixtieth hour. The amount of monosaccharides shows an appreciable descent corresponding to the respiratory drift and so does also the disaccharide content but slightly.

GENERAL DISCUSSION

1. Comparison of the CO_2 and sugar values.

It has been seen previously that the CO_2 values begin at higher level and gradually come down except in cases where factors connected with the ripening or the senescence of the fruit influence the respiratory drift. The descent of the respiratory curve probably implies starvation. As in the detached fruit the store of substances to be utilised

in metabolic process is rather limited, to continue respiring at the initial rate would mean a speedy depletion of the materials. And as simpler sugars are the substances ordinarily used in oxidative processes, a high respiratory rate would naturally lead to a rapid hydrolysis of the higher carbohydrates. The drain on the limited store would make its consequence felt and therefore respiratory rate would necessarily come down. Thus the gradual fall of the the CO_2 output from the initial values indicates this adaption to changed conditions of oxidative materials in time and the almost constant rate that follows it synchronises with physiological balance between higher carbohydrates to simple sugars and their consequent oxidation.

Now, as reducing sugars are utilized in respiration, the amount of such sugars lost during a certain time should give an indication of CO_2 evolved. But the amount of CO_2 calculated from the quantity of sugar lost during each experiment falls far short of that of the CO_2 actually given out, except in experiment No. 7. Similarly, the loss in calculated amount of sugar from the actual quantity of CO_2 evolved during each experiment is far in excess of the observed loss of sugar. The following table (No. 1) will make it clear :

TABLE I

Experiment No.	Total amount of sugar lost per gram of material from observation		Calculated amount of sugar per gram of material from CO_2 given out.		Total amount of CO_2 given out per gram of material from observation		Calculated amount of CO_2 per gram of material from loss of sugar.	
1	6	Mgs	12·81	Mgs	18·79	Mgs.	6·8	Mgs.
2	2·0	„	12·34	„	18·1	„	4·3	„
3	6	„	10·65	„	15·65	„	8·8	„
4	4	„	9·65	„	14·15	„	5·2	„
5	6	„	9·46	„	13·88	„	8·8	„
6	10	„	15·70	„	23·03	„	14·6	„
7	22	„	15·42	„	22·62	„	32·2	„

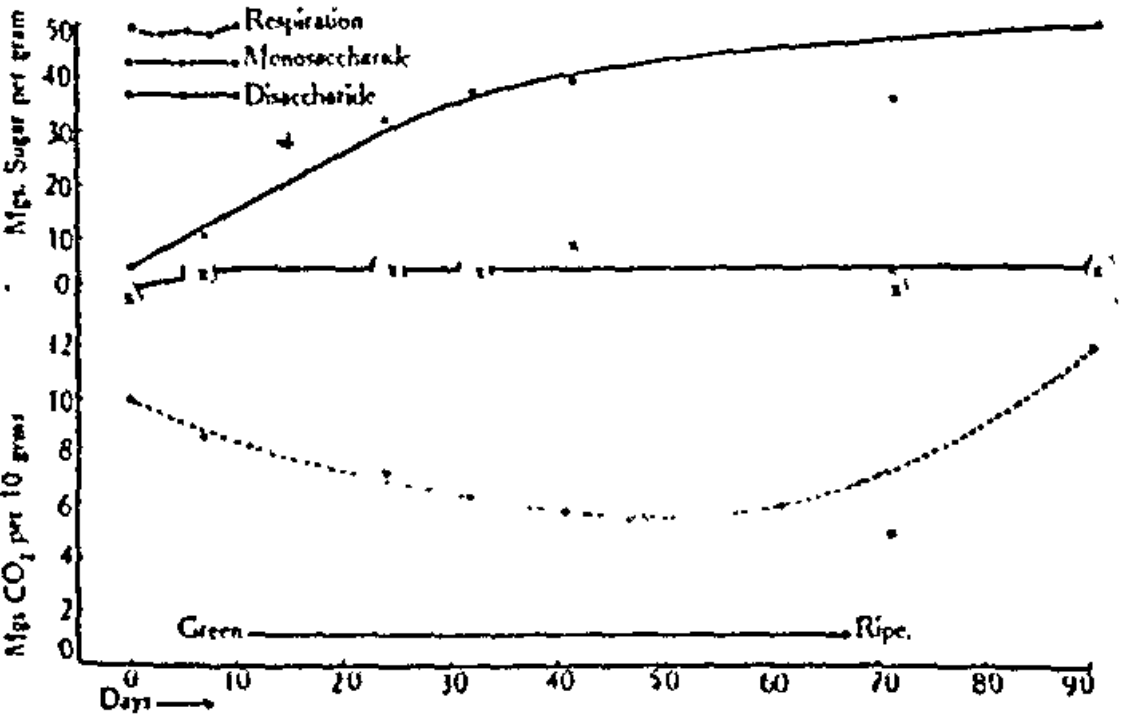
This anomaly is explained by the constant hydrolysis of the higher to lower carbohydrates, and thus making good the loss of the latter by respiration.

2. The drift from the green to the senescent phase.

The initial respiratory and monosaccharide and disaccharide values of all the experiment are plotted in the following graph (No. 8). The various curves have been correlated to the age of the fruit, in the following way :

The age of the fruit with which the first experiment was started is taken to be zero. It becomes then easy to represent the age on the abscissa as the number of days that separate two consecutive experiments is known. It will be seen that the respiratory drift begins at a high level with the green and immature fruit. It gradually descends throughout the

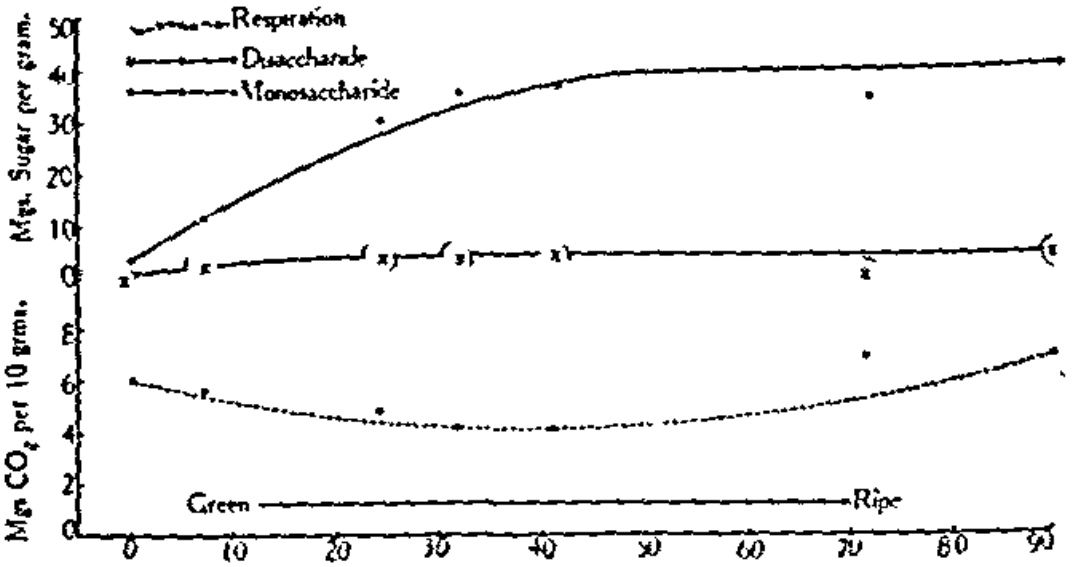
Graph No. 8.



period the fruit remains green but ascends about and at the time of ripening. While on the other hand, from the immature

to the senescent phase the monosaccharide value continues to go up and the disaccharides increase for some time and then remain constant. That the CO_2 output continues to go down and the monosaccharides go on accumulating is strange in view of the fact that the latter are the chief materials used in respiration. The possible explanation would probably come from what Blackman and Parija have aptly termed as "organisation resistance"—the protoplasmic control over the metabolic flux. Or, it may be that some other internal factor, probably the respiratory enzymes, is limiting; or the various products of the biochemical reactions within the maturing fruit act as depressant. It may be that these

Graph No. 9.



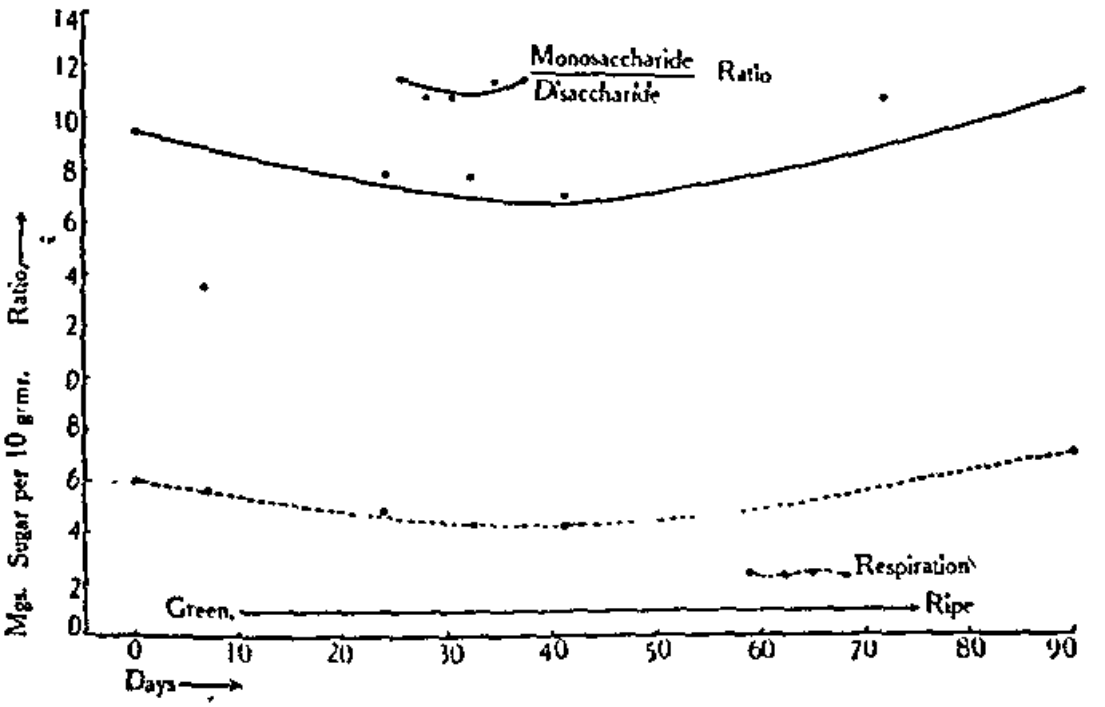
together hold the oxidative process in check, or that singly they are of varying importance at one or other phase of the ontogeny of the fruit. At the time of ripening the respiratory drift once again goes up. This is as Blackman and Parija suppose due to the lowering of organisation

resistance or the lowering of protoplasmic grip over the metabolic reactions. The same condition of things may be seen in graph No. 9 where, instead of the initial data as in the previous case, the average values of the carbon dioxide and disaccharide and monosaccharide contents of each experiment are plotted.

3. The $\frac{\text{monosaccharide}}{\text{disaccharide}}$ ratio and the respiratory drift.

In the following graph (No. 10) the average CO_2 values and the mean $\frac{\text{Monosaccharide}}{\text{Disaccharide}}$ ratio have been plotted. Both the respiratory and ratio curves correspond with each other—the latter like the former starting high gradually comes down and then ascends. When respiration is high

Graph No. 10.



more of simpler sugars should be present than the higher ones and so the ratio is high in the beginning and towards the ripening phase of the fruit. Between these two the ratio varies according as the respiration is high or low.

SUMMARY

1. A study of the respiration and monosaccharide and disaccharide contents of the fruit of Carica papaya from the immature green to the senescent phase has been undertaken.

2. The respiratory drift is high in the immature green stage, gradually falls during the mature green and rises again in the senescent phase.

3. The monosaccharide content is low in the beginning and rises gradually throughout the mature green and senescent phases.

4. The disaccharide content is very low at first, rises for some time and then remains constant.

5. The $\frac{\text{Monosaccharide}}{\text{Disaccharide}}$ ratio follows closely the respiratory drift.

I must thank Dr. Ranjan for his kind help and criticism.

LITERATURE

1. Archbold, H. K.—Chemical Studies in the Physiology of Apples. IX. Ann. Bot., Vol. 42, 1928.
2. Blackman, F. F.—Analytic Studies in Plant Respiration. III. *Proc. Roy. Soc.*, B., Vol. 103, 1928.
3. Blackman, F. F. and Parija, P.—Analytic Studies in Plant Respiration. I. Proc. Roy. Soc., B., Vol. 103, 1928.
4. Carre, M. H.—Chemical Studies in the Physiology of Apples, IV, Ann. Bot., Vol. 39, 1925.
5. Emmet, A. M.—An investigation on the changes which take place in the chemical composition of pears *stored at different temperatures with special reference to the pectic changes.* Ann. Bot., Vol. 43, 1929.
6. Evans, D. I.—Chemical Studies in the Physiology of Apples. X. Ann. Bot., Vol. 42, 1928.
7. Haynes, D.—Chemical Studies in the Physiology of Apples I. Ann. Bot., Vol. 39, 1925.

8. Kostychev, S.—Plant Respiration.
9. Luthra, J. C.
and Chima, J. S.—Some Studies in Respiration and Other Metabolic Activities in Berries of the Grape. Ind. Jour. Agri. Sci., Vol. I, 1931.
10. Parija, P.—Some Aspects of Respiration in Higher Plants. Presidential Address, Section Botany, 17th Indian Science Congress, Allahabad, 1930.
11. Parija, P.—Analytic Studies in Plant Respiration II. Roy. Soc., B., Vol. 103, 1928.

SECTION V

ZOOLOGY

ON THE CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF PERIPLANETA AMERICANA LINN*

BY

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CONTENTS

1. INTRODUCTION

The present problem, suggested by Professor Bhattacharya, was undertaken with a view to verify the observations recorded on the same animal by Hogben (36), V. Nath and P. Mohan (55), and to ascertain if the follicle cells contributed any of their Golgi contents to the developing oocyte.

The believers in the "Golgi" infiltration theory, advocated by Professor Bhattacharya and his pupils and Dr. Bramhell, aim at bringing to light the fact that the egg receives a

* Thesis submitted in lieu of two papers for the M.Sc. degree.

fresh quota of Golgi elements from the follicle cells in the manner described in this paper, and as in the case of the nutrient materials of Waldeyer (70) and Loyez (39), a possibly important physiological rôle is being played by these bodies during the development of the egg.

To elucidate the true nature of the cytoplasmic inclusions during oogenesis, Intra-Vitam observations by fresh cover-slips preparations were also resorted to and the results of the fixed preparations were confirmed by these Intra-Vitam observations. Both the methods having been tried, the confirmatory observations have been recorded here. The experiments have been carried out repeatedly and the figures that are given at the end of this paper represent typical appearances for each stage.

Here I take the opportunity of expressing my deep sense of gratitude to Professor Bhattacharya, under whose guidance this work was carried out.

2. PREVIOUS WORK

Hogben ('20) in his studies on synopsis (36) describes the formation of albuminous yolk in the oogenesis of Periplaneta americana from nucleolar extrusions. He describes two kinds of nucleolar extrusions. In the oogonia the nucleolus is in the form of plasmosome which in early oocytes emits minute deeply staining particles in the cytoplasm which migrate to the periphery of the oocyte where they are either ejected or transformed into some material no longer distinguishable from the ground cytoplasm itself. When this has completely ceased, at a certain point which has a definite relation to the deposition of yolk the plasmosome loses its opacity and becomes vacuolated. At a later stage these vacuoles become granular and acquire a more chromatic form. The vacuolar bodies within the plasmosome are cast out and make their way through the nucleolar membrane to the periphery of the egg. As these

intranucleolar bodies which may be termed deutosomes are discharged, new vacuoles appear within the plasmosome, until the deposition of yolk in the periphery of the egg is nearing completion. Hogben has simply noted the presence of mitochondria without assigning to them any function. So far as the Golgi bodies and their relation to the process of vitellogenesis are concerned he does not make even a passing mention about them.

V. Nath and P. Mohan in their work on oogenesis of Periplaneta americana (55) are in substantial agreement with the observations of Hogben so far as the nucleolar activity is concerned. These workers like Hogben only make mention of mitochondria and state that there is nothing interesting about mitochondria. Unlike Hogben they have studied the Golgi bodies and their relations to the process of vitellogenesis in the production of fatty yolk bodies. They have also described certain "bacterioids" which always lie at the periphery of the egg.

Very recently (Jan. '31) Gresson has published a paper on yolk formation in Periplaneta orientalis (32). In this work no mention of mitochondria is made at all. Golgi vesicles are said to give rise to fatty yolk bodies, and in neutral-red preparation during intra-vitam observations are said to appear as non-stained vacuoles developing an osmiophilic rim on introducing a few drops of 2 per cent osmic acid under the cover slip. Albuminous yolk is said to arise from the nucleolar extrusions, two types of which have been described. The nucleoli of the early oocytes are said to be amphiphilic or slightly basophil giving out nucleolar extrusions which are strongly basophil. These disappear, probably dissolve in the ooplasm. Their substance at a later stage probably contributes in some way towards yolk formation. The second type which appears later is also basophil. These become vacuolated and undergo fragmentation to form small dark granules. These are dissolved in

the cytoplasm and their substance is said to form clear vesicles which increase greatly in size and form the albuminous yolk globules.

Thus these observations are in general agreement with those of Hogben; V. Nath and P. Mohan on Periplaneta americana. Gresson, like V. Nath and P. Mohan, further describes "Bacterioids" in the ooplasm at the periphery of the older oocytes. He also states that the oocyte nuclei, nucleoli, and nucleolar extrusions are devoid of chromatin.

3. MATERIAL AND TECHNIQUE

The material for the study of the cytoplasmic inclusions in the oogenesis of Periplaneta americana Linn consisted of ovaries from young specimens. In the female a pair of ovaries are situated in the hinder part of the abdomen below the intestine and embedded in fat and haemocoelic packing tissue lying on their surface. Each ovary consists of eight threads which show an anterior tapering and beaded appearance due to a row of ova. Each bead contains one ovum. The eight threads of ovary are attached by means of a terminal filament to the wall of the body cavity anteriorly. The size of the ovarian threads and the beads depends upon the maturity of the female. In a full grown female specimen they are of conspicuously large size greenish or brownish in appearance.

The specimens were killed by severing the head quickly by means of a pair of scissors. Great care was taken to lose as little time as possible between the killing of the specimen and the subsequent fixing of the ovaries, to avoid as far as possible any post-mortem changes.

Nearly all the female specimens dissected in the first instalment in August and September were well grown mature females. As a result of this, early stages of eggs were difficult to get. But the females from the second instalments in January possessed ovaries which were not

in a very advanced stage of development, and all the stages right down from the oogonial to the fairly advanced oocyte could easily be found. This implies that the months of September and October are the breeding seasons of these animals. Satisfactory results showing the early stages of the oogenesis were naturally obtained from the material obtained in winter.

The following fixatives were used :

For the demonstration of Golgi bodies—DaFano's cobalt nitrate formol method and Ludford's latest method of the modified Mann-Kopsch's corrosive osmic fixative, were found to be very satisfactory in bringing out the desired results.

In silver fixatives both toned and untoned preparations were made. This process of toning which consists in treating the sections with 2 per cent gold chloride solution was introduced first by Golgi in 1908. In this process the reduced silver is replaced by gold while the excess of unstable silver still present in the sections is dissolved out. The slides are then treated with 5 per cent sodium hyposulphite solution. This fixes the substituted gold, and dissolves out the remaining unstable silver. The slides were stained with Iron-haematoxylin and Gatenby's safranin (saturated solution of safranin in aniline water and in absolute alcohol in the ratio 1 : 1) and light green stains.

In osmic fixatives both bleached and unbleached preparations were made. The process of bleaching consists in oxidising sections to such a point that the general precipitation of osmic acid in the cytoplasmic back ground is removed without serious loss of colour from the more blackened Golgi bodies. This process was first introduced by Veratti in 1908. The bleaching was done by Henneguy's method by employing 1 per cent $KMnO_4$ solution and the slides were then treated with 4 per cent oxalic acid to remove all traces of oxidising reagent—$KMnO_4$. The sections were as usual stained in Iron-haematoxylin and Champy-Kull triple stain

(5 per cent acid Fuchsin, ·25 per cent Toluidin blue, ·5 per cent alcoholic Aurantia and absolute alcohol).

For the demonstration of mitochondria modified formula of Champy-Nassanow, Regaud-Tupa's fluid and Regaud's formol bichromate method, were the processes employed. Generally speaking all of them were found to be very successful giving very satisfactory and convincing results.

Champy-Nassanow's method and Regaud-Tupa's fluid, however, might specially be emphasised as giving excellent results. In Champy-Nassanow fluid both kinds of yolk bodies were very nicely fixed besides mitochondria.

After Regaud's fixation post-chroming is necessary because by soaking tissues in $K_2Cr_2O_7$ one produces stainable compounds of cell protein and lipoids which may not be easily dissolved out by alcohols, and clearing oils.

Bouin's picro-formol and Carnoy's fluid were employed to study and demonstrate the nucleolar extrusions.

The sections were stained with Iron-haematoxylin and Mann's methyl blue eosine. The latter stain proved successful in so far as it left the cytoplasm clear without bringing about its granular structure.

All the stock solutions of the reagents were freshly prepared with scrupulous cleanliness and the fixatives were prepared only when needed.

For the Intra-Vitam examination neutral-red, Janus green B and 2 per cent osmic acid were employed. Neutral-red and Janus green B were freshly prepared 24 hours before use and kept for ripening at 35°C.

A few female specimens were fed with neutral-red mixed with cheese and also with some syrup placed on the slices of bread. Such specimens were found not to live long. The neutral-red feeding apparently seemed to have no effect on the ovaries.

4. OBSERVATIONS

(*A*) *Intra-Vitam Examination.*—Ovaries were taken out and placed in very dilute neutral-red solution (four drops of the stock solution added to 50 c.c. of physiological salt solution) for about 20 minutes. They were then examined under oil immersion lens and strong light. In a young oocyte a patch of pink-coloured body was observed near the nucleus (Fig. 30, V). This patch, it was observed, consisted of about five to six small separate vacuoles which apparently had run together. These bodies were recognized as Parat's vacuome or Gatenby's vacuoles. In a slightly older oocyte two such smaller patches of vacuome were observed. In young oocytes these pink-coloured vacuome occupy a juxta-nuclear position (Fig. 31, YNV) which in the fixed preparations is occupied by the Golgi bodies and mitochondria forming the yolk-nucleus of Balbiani. In the next stage the number of vacuome increased and discrete individual vacuome were also seen in addition to the big patches of vacuome. In later stages the number of vacuome increases and in the older oocytes they are found to be uniformly distributed (Fig. 32, V, V'). In addition to these pink-coloured vacuome some unstained refractory bodies were also found. These bodies, on the introduction of two per cent osmic acid under the cover slip, showed a black rim around a colourless core after about ten minutes (Fig. 33. FYB.); these were identified as the fatty yolk bodies. Distinct Golgi bodies also appeared in close proximity to the areas occupied by vacuome. The Golgi bodies had clearly vesicular structure with osmiophilic rim and central osmiophobic area. Some of the Golgi bodies were also crescent-shaped. I could not, somehow, meet with crescentic Golgi bodies in the fixed preparations. Neither Hogben, nor V. Nath and P. Mohan, or Gresson has observed vacuome on staining the material with neutral-red. Moreover, the latter two observers have also not seen crescentic Golgi bodies about

the existence of which no doubt exists in the present case. According to Y. Nath such crescentic forms are artifacts due to the improper osmic impregnation or optical sections. But if this is so, at no time do the crescentic Golgi bodies disappear howsoever long the osmication is continued. Unlike Y. Nath and P. Mohan, I do not find Golgi bodies stained with neutral-red.

Fresh ovaries were also treated with two per cent osmic acid only for about 20 minutes and were examined. Crescent-shaped and vesicular Golgi bodies appeared clearly (Fig. 34, C. G. B. & V. G. B.). This confirmed the previous observations that the crescentic forms are not the artifacts as a matter of partial impregnation but they do really exist as such. Fatty yolk bodies appeared as highly refractory bodies The distribution of Golgi bodies as seen in the Intra-Vitam observations further confirmed my conclusions arrived at by the aid of fixed preparations. Their distribution also agreed with the distribution of vacuome already stated. This led me to the conclusion that the vacuome are intimate associates of the Golgi elements. This has been shown by Gatenby (24) and others in a number of cases. In the oogonial stages, which were observed in the terminal filament of the ovarian thread two or three distinct Golgi granules were seen lying in the vicinity of the nucleus.

Fresh ovaries were also treated with Janus green B solution (4 drops of stock solution added to 50 c.c. of physiological salt solution) for about 10 minutes to see mitochondria. They appeared as dust—like granules stained green (Fig. 35 M). At the periphery of the oocyte and near the nucleus, baton-shaped mitochondria were also observed and some of them were even seen breaking up into small granules to form fine dust like granular mitochondria of the advanced oocyte. None of the previous workers has made use of this vital dye for the demonstration of mitochondria. Gresson—as has already been mentioned—does not even

mention mitochondria in his observations on this animal. I have not been able to see the breaking up of baton-shaped mitochondria in my fixed preparations. In the same preparation were also observed some refractory bodies. These were identified as albuminous yolk bodies which do not take either of the two vital dyes. Their distribution agreed with that observed in the fixed preparations. After about 20 minutes, one striking thing attracted attention. Some vesicular bodies began to appear in the same preparation in the places occupied by the Golgi bodies as seen in the fixed preparations and by the Intra-Vitam examination of the oocytes in 2 per cent osmic acid. These were recognised as the Golgi bodies (Fig. 36, G. B.). It is a well-known fact that Golgi bodies come into view after prolonged treatment with Janus green B. Bacterioids were also noted in the fresh preparations. They are very numerous and are situated at the periphery of the ovum.

(*B*) *Fixed Preparations.*—*Golgi Bodies.*—In an oogonium there are seen in the DaFano preparations stained with safranin light green two or three granular Golgi bodies in the cytoplasm which is almost clear and homogeneous (Fig. 1, G.B.). At this stage the nucleus occupies almost the entire space of the oogonium. As this oogonium develops to form an early oocyte the cytoplasm near the nucleus carrying the Golgi bodies becomes dense and is differentiated into a juxta-nuclear area called the "Yolk-nucleus of Balbiani" or simply the "Yolk-nucleus" of D'Hollander, Gatenby, Bhattacharya, and others (Figs. 3 & 9, YN.). This dense area takes stain more readily standing in sharp contrast to the rest of the cytoplasm of the oocyte. In the subsequent stages it acts as a focus of growth and dispersal of the Golgi bodies. It is very difficult to make out the exact shape and nature of the Golgi bodies in this area due to their being closely packed up and sometimes due to the extreme blackening by silver nitrate or osmic acid impregnations. In a

slightly advanced oocyte (Fig. 10) a peculiar and an unusual feature presents itself. Here two juxta-nuclear aggregations of Golgi bodies lying on opposite sides may be seen. Whether both of these originate independently or the second is a part of the older specialised area (Yolk-nucleus) it is difficult to say in the absence of any intermediate stages. Brambell (12) in the fowl has described several such areas in an oocyte of a six weeks old chick and describes them as an abnormal arrangement of the Golgi bodies. This peculiar feature has also been observed in the oogenesis of Calotes versicolar by Dutta and Asana (16) in this Laboratory. This stage is followed by a dispersal stage when the Golgi bodies become irregularly distributed in the cytoplasm either singly or in patches (Figs. 4, 5, 11 & 12, G. B.). By now the process of fatty yolk formation is fairly advanced. By bleaching the slides carefully it is possible to distinguish between the fatty yolk and the Golgi bodies. It has been found that a regular process of infiltration of the Golgi bodies from the follicular epithelium cells to the cortical region of the oocyte has been going on (Fig. 13, INF). The Golgi bodies from the follicular epithelium cells are transported to the periphery of the oocyte. The cell membranes of the follicular cells are very fine and delicate and offer no great resistance to the Golgi bodies in their infiltering process. In this case, however, the process of infiltration does not take place through any special area such as canalicular process in the zona radiata as described by Bhattacharya (3), in tortoises and reptiles (6) and certain other animals (2) and by Das in birds (18). The process is akin to that described by Brambell (12) in Gallus bankiva in which case the infiltration takes place before the formation of "zona pellucida."

The Golgi bodies settle down in the cortical region and form big lumps. Whether these lumps are the result of Golgi granules running together due to the influence of the

fixatives or as a result of their growth, I am unable to tell. These bodies, however, in good preparations give an idea that the former alternative is more probable. In the very advanced oocyte the Golgi bodies are generally distributed almost uniformly all over the ooplasm (Figs. 6 & 12). Here the fatty yolk formation is at its zenith and the egg is full of fatty yolk bodies. It appears that the majority of the Golgi bodies has been used up in the formation of fatty yolk. The remaining ones probably break up and become ultra-microscopic and appear to play no part in the subsequent development of the oocyte.

Mitochondria.—In an oogonium in which nucleus occupies almost an entire area three or four mitochondrial granules are seen in the vicinity of the nucleus (Fig. 15, M.). In an early oocyte the mitochondria appear to lie in the juxta-nuclear archoplasmic area (Fig. 16, Y.N.), in the same manner as the Golgi bodies do. I am inclined to think that both of these cytoplasmic inclusions—Golgi bodies and mitochondria—in the early stage of the development of an oocyte occupy the same area known as the "idiosome," or the "Yolk-nucleus of Balbiani," or "archoplasmic area," which functions as the focus of growth and dispersal for the Golgi bodies as well as for the mitochondria. The idiosome area disappears and along with it *the dispersal* of mitochondria takes place. *They become distributed in the ooplasm either singly or in patches forming* mitochondrial clouds (Figs. 19 & 23, M.). They have a pronounced tendency to become arranged in a fashion forming a nuclear cap (Figs. 17 & 24, N. C. M.). In addition to fine dust-like *granular mitochondria, baton-shaped mitochondria are also* found in the cytoplasm (Fig. 18, B. S. M.). In the highly advanced oocyte the mitochondria are distributed throughout the cytoplasm and patches of mitochondria are distinctly visible (Fig. 25). The albuminous yolk bodies begin to make their appearance from the stage when the

mitochondria form a nuclear cap. Sometimes the mitochondria seem to run together and form a lump or they may swell up individually (Figs. 19 & 25, S. M.) and become transformed into yolk bodies. In a still later stage the mitochondrial patches disappear and the mitochondria are evenly distributed throughout the ooplasm (Figs. 18 & 25, M.).

Nucleolar Extrusions.—There are no traces of the nucleolar extrusions—at least in the solid form. This is very clearly demonstrated by Bouin preparations stained by Mann's methyl blue eosine (Fig. 29). As such they do not make any visible contribution towards vitellogenesis. The same thing was further confirmed by examining the Champy-Nassanow preparations (Figs. 24, 25, 26). Material fixed in Carnoy's fluid also confirmed the same observations after staining by Mann's methyl blue eosine (Fig. 14). The nucleus, however, besides a prominent nucleolus contains a number of small nucleoli and a certain amount of chromatin; but none of these is ever seen to come out of the nuclear membrane in the cytoplasm at any stage of development of the oocyte. All these structures are strongly chromophilic. The nucleolar extrusions in the liquid form, may, however, exist.

Yolk Bodies.—Two kinds of yolk bodies are distinctly visible—(*i*) Fatty yolk bodies, (*ii*) Albuminous yolk bodies. The former are more easily blackened by osmic acid and so also more readily bleached than the Golgi bodies. This fatty yolk of Golgi origin can be distinguished from the ordinary fat droplets which are easily extractable in turpentine whereas the fatty yolk bodies are more resistible. They leave behind them a dark rim even after they have been completely bleached. This rim is the remnant of the osmiophilic Golgi element. In the Ludford preparations the fatty yolk bodies are seen to make their appearance with the establishment of the yolk nucleus. The dispersal of the Golgi bodies and their accumulation in patches in the

cytoplasm are accompanied by the formation of fatty yolk in these areas. With the dispersal of the Golgi bodies throughout the cytoplasm fatty yolk bodies appear to fill up practically the entire cytoplasm (Figs. 4, 6, 11 & 12, F.Y.B.). On close examination under the oil immersion lens it is found that these fatty yolk droplets undergo development in close association with the Golgi bodies. It is, however, difficult to say whether the Golgi bodies undergo direct transformation into fatty yolk bodies by drawing in the necessary requisites for their growth from the surrounding cytoplasm or whether in some indirect way they contribute towards the formation of these fatty yolk bodies. The fact that the Golgi bodies are solely responsible for the formation of "fatty droplets" (Ludford) or "fatty yolk" (Gatenby, V. Nath, and Bhattacharya) is borne out by the facts (*i*) that they (fatty yolk bodies) begin to make their appearance *pari passu* with the growth and dispersal of the Golgi bodies from the idiosome area, and (*ii*) that the Golgi bodies disappear from the view as the fatty yolk bodies are being fully formed.

Albuminous yolk bodies are apparently few. They make their appearance at a stage when the yolk-nucleus of Balbiani disappears and the fatty yolk is fairly well established. I could make out two methods of the formation of Albuminous yolk—(*i*) A process of direct metamorphosis of the mitochondria. In this case the granular mitochondria swell up in size by the absorption of the necessary materials from the cytoplasm (Figs. 18, 19, 25, S.M.) and ultimately give rise to the albuminous yolk spheres (Figs. 19, 25, A.B.Y.). (*ii*) A process in which a mitochondrium swells up to a larger size than its normal one and is encircled by a vesicle arising in the cytoplasm. Growth proceeds inside the vesicle in the usual manner by the absorption of necessary material from the cytoplasm (Fig. 25, A.B.Y.). The yolk body thus formed seems to lie in a sort of vacuole.

I could not see any trace of cytoplasmic yolk formation under the influence of mitochondria which has been described by Bhattacharya and Lall (3), and Bhattacharya and Mathur (4). The former authors state that when fully formed there is no way of distinguishing between the mitochondrial yolk and the cytoplasmic yolk as both of these follow the same fixing and staining reactions. Thus the only criterion to determine the presence of cytoplasmic yolk would be the formation of yolk vesicle under the influence of the smaller granular mitochondria which would surround such a vesicle and in the middle of which yolk would be deposited. In the absence of such definitive stages in my preparations it becomes difficult to distinguish clearly between the albuminous yolk of mitochondrial origin and that of the cytoplasmic origin.

(*C*) *Centrifuged Material.*—The ovaries of cockroach were centrifuged in the physiological salt solution immediately after being removed from the body of the animal. Curiously enough in this animal centrifuging for three hours at the rate of 3,000 revolutions per minute had no effect at all on the disposition of the cytoplasmic inclusions. The centrifuging was, therefore, prolonged to five hours in another experiment working at the velocity of 3,000 revolutions per minute. Here also, though the cytoplasmic inclusions were affected by the centrifugal force, the results obtained were not ideal. The centrifuged material after five hours' treatment was fixed in DaFano and Champy-Nassanow fluids. The usual technique was followed. Figure 27 shows the Golgi bodies accumulated at one pole, and a few of them distributed at the periphery of the oocyte. The mitochondria are accumulated at the opposite —the inner or centripetal—pole of the oocyte with clouds forming a perinuclear ring of mitochondria. The nucleus migrates more to the centrifugal or outer pole from the centre of the oocyte. In the Chamy preparation of the

same material (Fig. 28) the albuminous yolk bodies occupy the outer or centrifugal pole. Below these are seen fatty yolk bodies. Then there is the nucleus and mitochondria all accumulated to the centripetal pole. The Golgi bodies are distributed at random between the yolk bodies of both the types.

Lyon (43) in 1907 and his successors found that when the animal eggs are centrifuged the cytoplasmic inclusions are dislocated and are grouped in parallel strata at right angles to the direction of force. The number and relative bulk of these strata vary considerably with the nature of the cytoplasmic components. Generally, there are three or four such strata. Their order of succession being, of course, determined by their specific gravity. In general, the yolk granules—the heaviest of the inclusions—collect at the outer or centrifugal pole, fatty substances apparently including many of the mitochondria at the inner or centripetal pole, while the main bulk of clear substance largely hyaloplasm forms a central zone in which lies the nucleus.

5. DISCUSSION

Golgi Bodies.—The earliest stage of egg contained two or three granular Golgi bodies. These Golgi bodies at this stage are so minute that it is really very difficult to make out their exact morphology. But on a very careful examination they can be made out as being vesicular bodies having a chromophilic rim and a hollow chromophobic area. Gatenby ('19) was the first to show the complex nature of Golgi dictyosome composed of chromophilic and chromophobic areas (26). The Golgi bodies having such structure have been described in a number of vertebrate and invertebrate animals by various observers such as V. Nath in Pheritima (51), Leech, Culex (50), Spider (49); by V. Nath and Mehta in Luciola (54), by V. Nath and P. Mohan in cockroach (55), by Gresson in certain saw-flies of the family

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tenthridinidæ (31), and cockroach (32), by Sharga in earthworm (66), by Rai in Oyster (65), by Hill in Dephnia. In vertebrates the vesicular nature of the Golgi bodies has been described by P. R. Bhattacharya in fishes (7), and house Geko (8), by Prof. Bhattacharya in reptiles (6), and uromastrix, by Datta and Asana in Calotes (16), by Bhattacharya and Lal in tortoises (3), and by Dr. Das in pigeon (18).

Various forms such as rod-shaped granules (Brambell), curved rods (Ludford), crescentic forms (Lepidosome of Parat and Dictyosome of Gatenby) and a number of others have been attributed to the Golgi bodies.

Originally Camillo Golgi described the apparatus that goes after his name as the "Apparato reticolare interno" meaning thereby that it has a reticular structure. Harvey and V. Nath have criticised all these forms as artifacts resulting by one of the following reasons. The curved rods or baton-shaped may either represent optical sections of spheres, or may possibly be due to the incomplete impregnation of the rim of Golgi vesicles. Granules have widely been accepted as resulting from the excessive osmication or silver impregnation. Whatever their form the Golgi bodies ultimately form vacuolar structures or become associated with such structures. It has been clearly demonstrated that rod-shaped Golgi granules in Helix (Brambell 11) are metamorphosed directly into vacuoles containing fat. Thus a rod-shaped granule is apparently converted into a vacuole. Curved Golgi rods in Patella (Ludford 40) are said to give rise to vacuoles from their archoplasm which soon break on the completion of the metamorphosis of the archoplasm into a vacuole. And sometimes the curved Golgi rods are also directly metamorphosed into yolk vacuoles. Moreover, Gatenby in his work on the Golgi apparatus and vacuolar system of Cavia, Helix, Abraxas, by Intra-Vital methods (24) suggests that the differences in

the production of vacuoles by Golgi bodies are only apparent and hence unreal. For in conclusion he also states that there exists in the animal cells a vacuole or a system of vacuoles primitively associated with and probably produced by the argentophil cortex of the Golgi apparatus. In the egg the vacuoles are closely related to the chromophilic substance of the Golgi apparatus which condenses in them small substances such as fat, lipin and zymogen (in glands). Such vesicular Golgi bodies would according to Parat correspond with his vacuome and the intensely osmicated or argentophil portion which would sometimes form the crescent-shaped Golgi body—dictyosome—would correspond with his Lepidosome—modified mitochondria. I have no doubt, however, that these forms are true Golgi bodies. At least so far as this animal is concerned it is difficult to maintain Parat's statement that the dictyosomes are the modified mitochondria, *i.e.*, Lepidosomes, as all the tests that are applicable to the Golgi bodies have been used with success in this case.

Subsequently the Golgi vesicles grow in number and size and begin to distribute. My observations differ from those of V. Nath and P. Mohan in Periplaneta americana so far as the distribution phases of the Golgi bodies are concerned. They observe three phases in the distribution—(i) circumnuclear ring, (ii) peripheral arrangement, (iii) uniform distribution. They also differed from Gresson (32) who agrees with the former workers in all essentials about the distribution of the Golgi bodies. I do not see these successive stages distinctly. After the breaking up of the yolk-nucleus of Balbiani, which has not even been mentioned either by V. Nath and P. Mohan (55) or by Gresson (32), the Golgi bodies begin to distribute without any circumnuclear or peripheral migrations. They do not also distribute uniformly until the very advanced oocyte stage is reached in which the whole of the oocyte is packed full of yolk bodies

(Figs. 6 and 12, F.Y.B.) leaving only a few Golgi bodies here and there. These may be said to be uniformly distributed otherwise their distribution in the oocyte is in groups and these form the Golgi patches. This distribution in patches is noteworthy and as such there should be some hesitation in attributing to them straight on a uniform dispersal.

During the development of the oocyte the follicular epithelium plays an important part at a certain stage, which has escaped the attention of the previous workers—V. Nath, P. Mohan and Gresson. A process of emigration of Golgi bodies from the follicular epithelium to the egg can be readily made out (Fig. 13, INF). The Golgi bodies do not pass through any definite passage as has been described by Bhattacharya (6); and Dr. Das (18); but in a haphazard manner described by Brambell (12). As I have worked with a large number of ovaries repeatedly I presume that the chances of artifacts are totally eliminated. Since these granules are indistinguishable from the original Golgi bodies of the oocyte and since they react to the fixatives in the same way as the Golgi bodies do inside the oocyte I infer that they must be identical bodies. What rôle these extraneous Golgi bodies might play in the physiological development of the egg I am unable to say at present.

The idea that granules could pass from the follicular epithelium into yolk of the egg is not a new one. Waldeyer (70) and Loyez (39) and a number of other authors admitted that granules pass from the follicular cells to the egg. Loyez states that the rôle of the large follicle cells in reptiles is to provide substances for the yolk formation to the egg, and that these substances that pass by way of canaliculi like prolongations may be fluids, semi-fluids, or granules. Thus the idea that Golgi bodies are extruded in the same way into the egg could easily be believed. Bhattacharya ('25) was the first to show that such a phenomenon actually exists (6).

Brambell (12) mentions that the apparatus in the follicular cells divides into two, only one-half being extruded into the oocyte. Whereas I find that fairly large number of granules assemble at the bases of the cells at a stage *preparatory to their infiltration through the membrane.* This is the period of intensive activity in the follicular epithelium layer of cells. The Golgi bodies now extrude from the cells of the follicular epithelium and are deposited in the cortical region of the oocyte. To my knowledge no infiltration of the Golgi bodies from the follicular epithelium cells has been so far described in invertebrates and as such it needs more critical study than I have been able to pursue.

The Golgi bodies in the subsequent development grow in size and give rise to fatty yolk. It is not possible to say at what stage of swelling and deposition of fat inside their interior the Golgi vesicles should be called fatty yolk bodies. In the Ludford preparation treated with turpentine (for 18 hours), which dissolves fatty yolk, the egg appears frothy due to the empty yolk vacuoles. The non-fatty Golgi vesicles which appear as solid spheres before now appear distinctly hollow with the characteristic osmiophilic rim and the central osmiophobic area. Bouin preparations (Fig. 29) also show the frothy appearance of the advanced oocyte due to the fatty contents being washed out by the acetic acid of the fixative.

In the oocytes centrifuged for five hours at 3,000 revolutions per minute the Golgi bodies occupy the upper or centrifugal pole and a few of them are also distributed at the periphery of the oocyte (Fig. 27, G. B.). It appears from this that the assortment of the Golgi bodies has taken place in two directions—(i) in the centrifugal, and (ii) in the peripheral direction.

In the intra-vitam examination unlike all the previous workers I find Golgi vesicles in the oocytes treated with Janus green B for about 20 minutes. I also find crescent-shaped Golgi vesicles in the intra-vitam observations which

I could not, somehow, meet with in the fixed preparations. My intra-vitam observations differ from those of V. Nath and P. Mohan, and are in agreement with those of Gresson so far as I do not see Golgi bodies stained with neutral-red.

Wilson has stated that in the ova of certain Hymenoptera (ants), Orthoptera (cockroaches) and Lepidoptera there are rod-like and rounded bodies, sometimes present in great number, dividing by fission, and variously known as "bacterioids" or "intracellular symbionts." Blochmann ('84, '87) who first observed these bacterioid forms in Blatta and Periplaneta found that in their earlier stages the oocytes are free from these bodies but are later infected with them from the surrounding cells. Lately these have also been isolated and cultivated by Glaser ('30) who states that the penetration of the egg by these bacterioids occurs after oviposition (30). V. Nath and P. Mohan and Gresson have found these forms always lying at the periphery of the egg which according to the former set of observers obscure in sections the inner membrane of the follicular epithelium. I have also noted the presence of these bacterioids lying at the periphery of an ovum in large numbers arranged in a regular manner.

Mitochondria.—Broadly speaking mitochondria are generally described as vesicular, baton-shaped, cup-shaped, filamentar or thread-like. Prominently hollow vesicular mitochondria have been described in male germ cells of scorpion by V. Nath (46). Marked difference in the size and the shape of mitochondria of male and female germ cells has been observed by Gatenby in Paludina vivipara. Filamentar mitochondria have been described in Emys lutaria by Bulliard; Bhattacharya and Lal (3) in tortoises; Bhattacharya and Mathur (4) in Pila globosa. In Columba intermedia cup-like dense clouds of mitochondria have been described by Dr. Das (18). P. R. Bhattacharya describes baton-shaped mitochondria in house Gecko (8).

The term mitochondria was invented by Benda and the granules now designated by this name were by earlier observers described as "microsomes." Benda applied this term to the granules in the sperm forming cells that were long ago described as "cytomicrosomes" by La Valette St. Georges ('86).

In the animal under observation I find spherical granules of mitochondria, rarely baton-shaped. They are very fine dust-like granules far less numerous in early stages. In a little later stage they increase in number and form mitochondrial cloud of yolk-nucleus in the juxta-nuclear archoplasmic or idiozomic area (Fig. 16, Y.N.). In an advanced oocyte they form a cap-like investment (Fig. 17, N. C. M.) at one pole of the nucleus. This is succeeded by a stage in a fairly advanced oocyte in which the mitochondria disperse and become distributed in patches (Figs. 19 & 23, M.). I have not been able to find a perinuclear ring of mitochondria described by V. Nath and P. Mohan (55) in Periplaneta americana. Gresson does not even make mention of these bodies. Bhattacharya in reptiles and Brambell in fowl have described a very striking perinuclear ring and medullary zone of mitochondria respectively. None of these stages I have been able to find in this animal. In an advanced oocyte mitochondria become very numerous, certainly by multiplying in some way or other which I have not been able to see in the fixed preparation. The growth of mitochondria from a very small granule to a fairly large swollen body is easy to follow with all the intermediate stages that are available. The mitochondria of the advanced oocytes ultimately metamorphose into the mitochondrial yolk bodies.

In the centrifuged egg (Figs. 27 & 28, M.) mitochondria are always seen to occupy the lower or centripetal pole with clouds forming a perinuclear ring of mitochondria.

In the intra-vitam observation they are stained brilliantly green by Janus green B diluted as already stated. All the

stages of dispersal that are observed in the fixed preparations are confirmed by the intra-vitam observations. None of the previous workers has attempted to observe mitochondria by intra-vitam examination.

My observations so far as the mitochondria in the oogenesis of this animal are concerned differ from those of V. Nath and P. Mohan in several matters of detail. These workers considered them as being of no particular interest. Whereas the fact that they definitely give rise to albuminous yolk makes them a very prominent and important cytoplasmic inclusion. In spider V. Nath (49) has described mitochondria as taking a fine red stain after treatment with neutral-red. I have great doubt if the neutral-red which is specific for vacuome only in an intra-vitam examination would stain anything but vacuome. The probabilities are that he might have confused neutral-red precipitations with the mitochondrial granules.

Nucleolar Extrusions.—Hogben (36); V. Nath and P. Mohan (55) and Gresson (32) working on Periplaneta americana and P. Orientalis respectively have laid great stress on the existence of the nucleolar extrusions and their relation to the formation of albuminous yolk during the vitellogenesis. The account given by these authors is no doubt very interesting. They describe two successions in the nucleolar extrusions. The products of the first activity, they state, disappear and are absorbed in the ground cytoplasm. The products of the second series of activity are of fundamental importance as they contribute towards the formation of albuminous yolk. The chromatic nucleus loses its opacity and develops vacuoles inside it, and gives rise to extrusions. These extrusions pass out through the nuclear membrane in the ooplasm and again become vacuolated in their turn like the mother nucleolus. These later on increase in number by breaking up and give rise to a third and final

series of bodies similar to them in all respects. These final extrusions are responsible for the production of albuminous yolk. These bodies, according to them, migrate to the periphery of the egg. This migration is due, according to V. Nath and P. Mohan, to the fact that the nutrient fluids flow to the egg from outside through the follicular epithelium cells and thus the periphery of the egg is, therefore, the most favourable place where the nucleolar extrusions can grow. These extrusions are directly metamorphosed into albuminous yolk bodies. This is in short the whole history of the nucleolar extrusions and their ultimate fate in the production of albuminous yolk described by the previous workers. Gresson has studied the phenomenon in greater detail ; in addition he has also studied the colour reactions of the nucleolar extrusions.

My material in spite of repeated experiments and observations failed to show any nucleolar extrusions. I made a very thorough search to see anything like that described by the previous workers, but all to no purpose. I find no evidence of nucleolar extrusions through the nuclear membrane. Figures 14, 26 and 29 will show that there are a few more bodies in the karyoplasm besides the nucleolus. But none of these is ever seen to come out as extrusions through the nuclear membrane. Bouin preparations stained with Iron-haematoxylin show very clearly granular nature of cytoplasm. To avoid every possibility of confusion between the granular cytoplasm and the nucleolar extrusions, if there be any, I stained Bouin preparations with Mann's methyl blue eosine only (Fig. 29) which did not bring out the cytoplasm as a granular ground substance but showed its more or less homogeneous structure with a beautifully stained nucleus and nucleoli. I failed to find any nucleolar extrusions even with this method. In order to confirm my results further I finally used Carnoy's fluid and stained the preparations with Mann's methyl blue eosine (Fig. 14) but

the result was again the same. I want, therefore, to state that my observations fundamentally differ from those of the previous workers in this respect. I have come to the conclusion that there are no nucleolar extrusions in this animal at least in the solid form. If there be any they might be in the liquid form or highly granular.

V. Nath has described the nucleolar extrusions giving rise to albuminous yolk in a number of other animals such as Euscorpius, Buthus (46), Luciola [V. Nath and Mehta (54)]; Lithobius by V. Nath and by King; in Scolopendra [V. Nath and Husain (53)]. Gatenby ('22) was the first to show clearly the exact relationship between the nucleolus and the process of vitellogenesis in the case of Saccocirrus (22). In this form he demonstrated the origin of albuminous yolk from the nucleolar extrusions directly. Nucleolar extrusions have been described in the case of Patella (Ludford 40); Limnoea, Helix and Patella (Gatenby and his pupils); tortoises (3), and mollusca (4) (Bhattacharya and his pupils) and in certain saw-flies of the family Tenthridinidæ by Gresson (31) they have also been described to give rise to fatty yolk bodies in Loris (N. Rao, 71).

Yolk Bodies.—Brambell has described four kinds of yolk bodies in oogenesis. He calls them—(*i*) Golgi yolk, (*ii*) mitochondrial yolk, (*iii*) cytoplasmic yolk, and (*iv*) the yolk formed by the nucleolar extrusions. These four kinds of yolk bodies may be classed into two groups according to their chemical nature, (*i*) those formed by Golgi bodies, and generally accepted as fatty yolk bodies, and (*ii*) those formed by mitochondria, cytoplasm, nucleolar extrusions or sometimes even by Golgi bodies and generally regarded as albuminous or proteid yolk bodies. Gatenby and V. Nath gave out for the first time the suggestion that there are essentially two kinds of yolk bodies in the oogenesis (*i*) fatty yolk bodies, and (*ii*) albuminous yolk bodies. Later on V. Nath in a series of papers has been emphasising the

fact that the fatty yolk bodies are essentially the products of the activity of Golgi bodies derived from them directly or indirectly. Instances are not wanting where the fatty yolk is formed from the direct or indirect activity of the Golgi bodies, *e.g.*, Gatenby has described the same thing in Saccocirrus (22), V. Nath and King in Lithobius; V. Nath in Julus, Palamnoeus (46); Culex (50); Spider (49); Luciola (54); Gresson in P. Orientalis (32); Brambell in Helix and Patella (11); Hirschler in Ciona and Bhattacharya and Parat in Ciona intestinalis (63); King in Oniscus asellus (38) and by Hibbard in Discoglossus (35). A number of other workers in different animals have described similar results. The idea is not a new one, for both Gatenby and Ludford had described previously the formation of yolk of a fatty nature from Golgi bodies. The same thing has also been described in this Laboratory by Dr. Das (18), Bhattacharya and his pupils (3 and 4), Dutta and Asana (16). I have also found the same thing in the present work. Gatenby, Woodger, Ludford and Brambell have described in Patella the indirect origin of fatty yolk from the Golgi bodies. The difference in the origin of fatty yolk does not in any way detract from the work of Gatenby and his pupils, who showed for the first time, that the Golgi elements play some part in vitellogenesis. Gatenby himself pointed out that re-investigation of this form (Patella) in view of Parat's claim might yield interesting results.

In the present case my observations are in complete agreement with those of V. Nath and P. Mohan on Periplaneta americana and Gresson on P. orientalis so far as the origin and nature of the fatty yolk are concerned. I believe that the Golgi vesicles grow in size and deposit fat in their osmiophobic vesicles. It is difficult to say exactly at what stage the swollen Golgi vesicles should be called fatty yolk spheres. These bodies in an advanced oocyte if decolourised in turpentine appear as

clear vacuoles leaving behind them a fine black rim. The Golgi vesicles, however, resist the action of turpentine and it is the core only which can be bleached out. The fact that fatty yolk bodies cannot be mistaken for fat vacuoles is proved thus: (*i*) that they are not easily decolourised in turpentine as fat vacuoles generally are, and (*ii*) when they are decolourised they appear still to leave a fine black rim which is reminiscent of their Golgi origin.

In the centrifuged egg (Fig. 28, F.Y.B.) the fatty yolk occupies the centrifugal pole below the strata of albuminous yolk bodies which forms the first and an outermost strata. In between these fatty yolk bodies are scattered Golgi bodies in different stages of their development.

In the *intra-vitam observations fatty yolk bodies* are seen as highly refractory bodies in the neutral-red preparations, with their rim deep black and the interior core rather greyish in two per cent osmic acid preparations.

So far as the formation of the albuminous yolk is concerned my observations differ from those of all the previous workers on cockroach in every minute detail. They attribute the origin of albuminous yolk to the nucleolar extrusions which are directly transformed into albuminous yolk bodies. I find that the albuminous yolk in this animal, in view of the total absence of nucleolar extrusions, is entirely derived from the mitochondria. There are, in my opinion, two ways by which the albuminous yolk appears. In one case the mitochondria grow in size (Figs. 18, 19, 25, S. M.) either singly or in an aggregate. They swell up to form pretty big masses which get directly metamorphosed into the albuminous yolk bodies (Figs. 19, 23, 25, A.B.Y.). In the second case, a vesicle in the cytoplasm gets formed and at the centre of this vesicle is lodged a single mitochondrium or a number of such very fine dust-like bodies (Fig. 25, A.B.Y.) which grow in size by swelling and elaborate the yolk material inside the vacuole. But this is very

rare. More generally the albuminous yolk is formed by the direct metamorphosis of the swollen mitochondria. The previous workers on cockroach are of the opinion that mitochondria do not at all play any rôle in the oogenesis much less in the elaboration of the albuminous yolk.

In the centrifuged material (Fig. 28, A.Y.B.) the albuminous yolk bodies occupy the extreme periphery of the centrifugal pole. In the intra-vitam examination they appear as solid refractory bodies in the material treated with Janus green B.

In this Laboratory, mitochondrial origin of albuminous yolk has been described by Bhattacharya and Lal (3) in tortoises; Bhattacharya and Mathur (4) in Pila globoso; Dutta and Asana (16) in Calotes, and by Das (18) in birds. Elsewhere the same thing has been described by Brambell (12) in fowl; by King (38) in Oniscus asellus; by Hirschler in Ciona; by Parat and Bhattacharya (63). In certain cases the origin of albuminous yolk is attributed neither to mitochondria nor to the nucleolar extrusions. In such cases it is said to be elaborated by cytoplasm. Such cases have been described by Ludford (40); V. Nath (49); by Gatenby and his pupils in Limnoea, Helix and Patella. Harvey in Carcinus (34) and Hibbard in the vertebrate Pygosteus and Discoglossus have described the origin of albuminous yolk from the Golgi bodies. In the latter two cases such Golgi vacuoles are described by Hibbard to be stainable with the neutral-red. Such Golgi bodies if compared with those that are not stained by the neutral-red and which give rise to fatty yolk, suggest that there might be some chemical difference in the nature of these two types of Golgi bodies.

6. SUMMARY

1. The oogenesis of the Periplaneta americana has been studied both by intra-vitam examination and fixed preparations.

2. In the oocytes treated with dilute neutral-red for about 20 minutes the vacuome is tinged red and gradually becomes prominent.

In the earliest oocytes there are one or two patches of vacuome consisting of about 5 or 6 discrete vacuome. With the growth of the oocyte these patches increase in number and are uniformly distributed throughout the oocyte. In the advanced oocyte separate discrete vacuome are also clearly visible among the large and prominent patches of vacuome.

3. The Golgi vacuoles are not stained by neutral-red and appear as refractory bodies which on the introduction of 2 per cent osmic acid under the cover slip clearly show the osmiophilic rim around a colourless core.

4. In the oocyte treated only with 2 per cent osmic acid for about 20 minutes the vesicular Golgi bodies with chromophilic rim and chromophobic core are clearly seen. There are also some crescent-shaped Golgi bodies.

In the youngest oocyte the Golgi bodies aggregate in a juxta-nuclear position forming yolk-nucleus of Balbiani. From now onwards they distribute in cytoplasm in patches.

5. With the growth of the oocyte the Golgi bodies grow in size and begin to deposit fat in their central core, and become converted into fatty yolk bodies.

Precisely similar stages of the Golgi bodies were observed in the fixed preparations.

6. Golgi bodies are also derived from the follicular epithelium cells of the oocyte by a process known as the "infiltration of the Golgi bodies" into the oocyte.

7. In the oocytes treated with a dilute solution of Janus green B for about 10 minutes the mitochondria are seen as fine granular bodies. There are some baton-shaped mitochondria also, some of which may be seen breaking into granular mitochondria.

In the youngest oocyte they are aggregated in the juxta-nuclear position to form the yolk-nucleus of Balbiani.

In advanced oocytes they are distributed in patches ; and ultimately they get evenly distributed in the cytoplasm as granular bodies.

8. Mitochondria, like Golgi bodies, swell up and directly give rise to albuminous yolk which in the intra-vitam examination is seen to be less refractory than the fatty yolk bodies and is slightly tinged with Janus green B.

Sometimes a single mitochondrium or a few granules are surrounded by a cytoplasmic vacuole where they grow in size by swelling and give rise to albuminous yolk.

Precisely similar stages of mitochondria were observed in the fixed preparations.

9. There is no evidence of nucleolar extrusions in the solid form.

10. The presence of bacterioids was also noted both in fixed and fresh preparations. They are in a very large number arranged at the periphery of the ovum almost regularly.

11. Centrifuge experiments gave the usual results.

12. After a prolonged treatment of the fresh ovary with Janus green B (for over 20 minutes) Golgi bodies appear in the intra-vitam examination.

LETTERING

A. Y. B. ... Albuminous yolk body.
B. S. M. ... Baton-shaped mitochondria.
B. S. M. divi. ... Baton-shaped mitochondria dividing.
C. G. B. ... Crescent-shaped Golgi body.
E. M. ... Egg membrane.
F. E. ... Follicular epithelium.
F. Y. B. ... Fatty yolk body.
F. Y. V. ... Fatty yolk vacuole.
G. B. ... Golgi body.
G. Y. ... Golgi yolk.
I. N. F. ... Infiltration of Golgi bodies.
M. ... Mitochondria.

N. ... Nucleus.
N. C. M. ... Mitochondrial cap of nucleus.
Nu. ... Nucleolus.
S. G. B. ... Swollen Golgi body.
S. M. ... Swollen *mitochondria*.
V. ... Patch of vacuome.
V'. ... Small discrete vacuome.
V. G. B. ... Vesicular Golgi body.
Y. N. ... Yolk-nucleus.
Y. N. V. ... Patch of Vacuome at the yolk-nucleus.

EXPLANATION OF FIGURES

(All the figures have been drawn with the help of Camera lucida.)

Figs. 1 to 6. Da Fano. Safranin light green.

Fig. 1. An oogonium showing three Golgi granules.
„ 2. An early oocyte showing Golgi granules.
„ 3. An early oocyte showing the yolk-nucleus of Balbiani.
„ 4. An advanced oocyte showing the distribution of Golgi bodies and formation of fatty yolk bodies.
„ 5. An advanced oocyte, later stage, showing the distribution of Golgi bodies in patches, the fatty contents of the Fatty yolk vacuoles have dropped out.
„ 6. Highly advanced oocyte packed full with yolk vacuoles. The Golgi bodies are scattered in between the yolk vacuoles.

Figs. 7 to 13. Ludford. Champy-Kull.

Fig. 7. An oogonium showing four Golgi granules.
„ 8. An early oocyte showing Golgi granules.
„ 9. An early oocyte showing the yolk-nucleus of Balbiani.
„ 10. An advanced oocyte showing two juxta-nuclear aggregations of Golgi bodies.
„ 11. An advanced oocyte showing the distribution of vesicular and granular Golgi bodies in patches, and fatty yolk formation.
„ 12. Highly advanced oocyte packed full with fatty yolk vacuoles. Golgi bodies are scattered in between the yolk vacuoles.
„ 13. A portion of the wall of an oocyte showing the infiltration of Golgi bodies (INF) through the follicular epithelium

Fig. 14. Carnoy. Mann's methyl blue. A group of three oocytes showing prominent nuclei and nucleoli. The homogeneous ground cytoplasm is represented by the grey background of the paper. There are a few more bodies besides the proper nucleolus in the nuclei, none of these bodies is seen to come out as the nucleolar extrusions.

Figs. 15 to 19. Regaud. Iron-haematoxylin.

Fig. 15. An oogonium showing two or three granular mitochondria.

„ 16. An early oocyte showing the juxta-nuclear arrangement of mitochondria forming the yolk-nucleus of Balbiani.

„ 17. An advanced oocyte showing the nuclear cap of mitochondria, N. C. M.

„ 18. An advanced oocyte showing the distribution of mitochondria, with a few baton-shaped and swollen mitochondria. B. S. M., S. M.

„ 19. An advanced oocyte, later stage, showing the distribution of mitochondria in patches, and formation of albuminous yolk by the direct metamorphosis of the swollen mitochondria.

Figs. 20 to 23. Regaud-Tupa. Champy-Kull.

Figs. 20, „ 21, „ 22 } Early oocytes with granular mitochondria distributed in the ooplasm.

„ 23. An advanced oocyte showing granular, swollen and baton-shaped mitochondria, and the formation of albuminous yolk by the direct metamorphosis of the swollen mitochondria.

Figs. 24 to 26. Champy-Nassanow. Champy-Kull.

Fig. 24. An oocyte with nuclear cap of mitochondria.

„ 25. An advanced oocyte showing swollen mitochondria. At places the swollen mitochondria are surrounded by a cytoplasmic vesicle in which they grow and metamorphose into an albuminous yolk body.

„ 26. A chain of five highly advanced oocytes showing albuminous yolk bodies at the periphery, mitochondria are distributed in the ooplasm. There are also seen fatty yolk vacuoles.

Fig. 27. DaFano safranin light green.

A centrifuged oocyte showing the upper layer of Golgi

bodies which are also distributed all round the periphery of the oocyte. Then there is a nucleus with mitochondria forming a perinuclear ring the majority of which are accumulated towards the centripetal pole. A few Golgi bodies are also seen among the mitochondrial aggregation.

Fig. 28. Champy-Nassanow. Champy-Kull.
A centrifuged oocyte showing the upper layer of albuminous yolk bodies followed by layer of fatty yolk bodies. In between these are scattered the Golgi bodies. Then there is a nucleus and below are mitochondria forming a nuclear cap.

Fig. 29. Bouin. Mann's methyl blue eosine.
A group of four oocytes showing the granular cytoplasm with fatty yolk vacuoles. The contents of which have been washed out. In the nucleus there are a few more bodies besides the nucleolus, but none of these is seen coming out as nucleolar extrusions.

Figs. 30 to 33. Fresh oocytes treated with neutral-red for twenty minutes.

Fig. 30. An early oocyte with a patch of vacuome consisting of 5 or 6 discrete vacuome.

,, 31. The juxta-nuclear arrangement of vacuome.

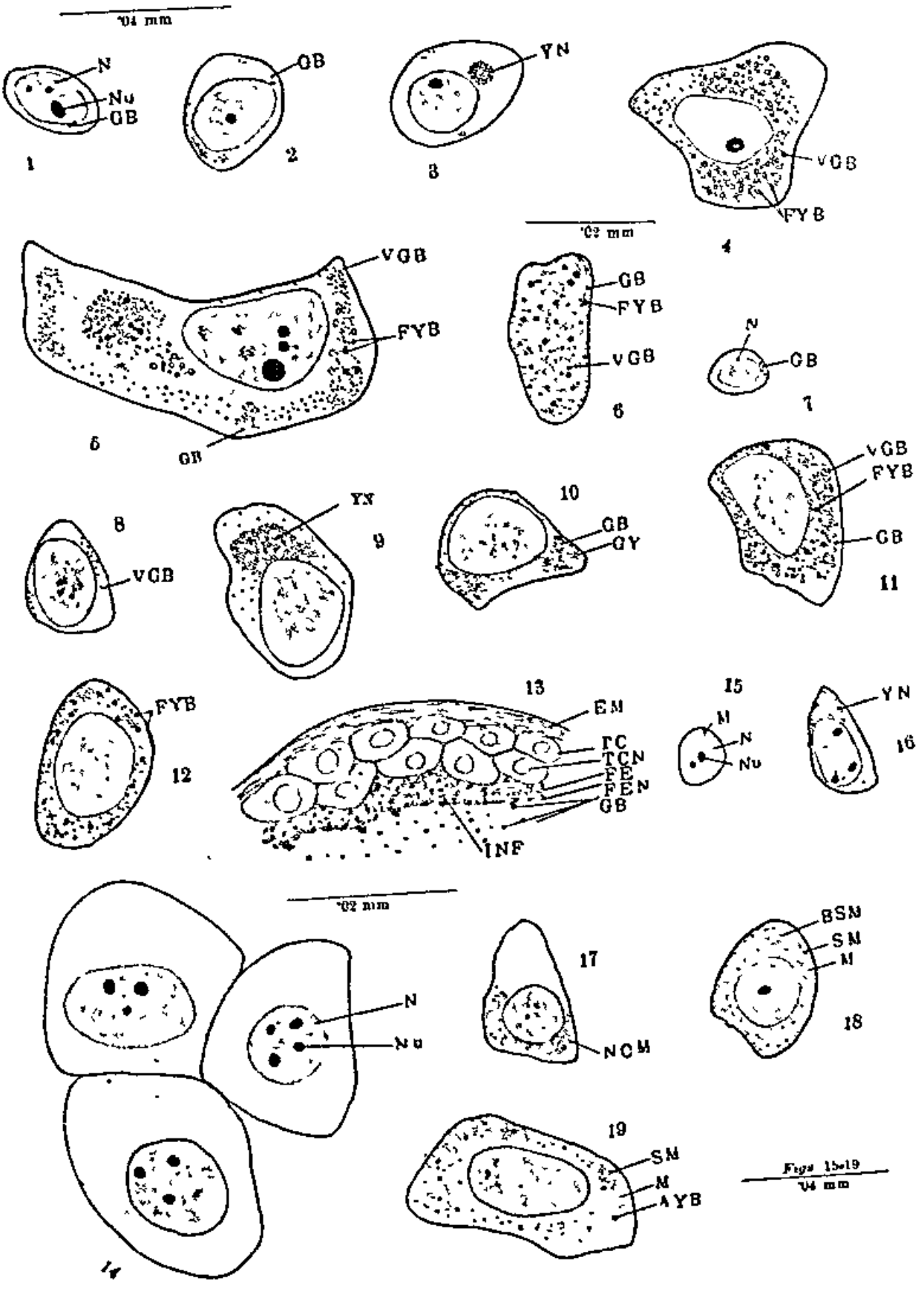
,, 32. The distribution of vacuome patches and discrete vacuome. Fatty yolk bodies are seen as refractory bodies non-stained.

,, 33. Showing the relation of the Golgi bodies with the vacuome. The material is treated with 2 per cent osmic acid after the neutral-red treatment. Fatty yolk bodies develop the osmiophilic rim of Golgi element. Golgi bodies also make their appearance in close connection with the vacuome.

Fig. 34. Fresh oocyte treated only with 2 per cent osmic acid for twenty minutes showing Granular, Vesicular and crescentic Golgi bodies distributed throughout the oocyte. The fatty yolk bodies are seen as highly refractory bodies with osmiophilic rim.

Fig. 35. Fresh oocyte treated with Janus green B for ten minutes. Showing granular and baton-shaped mitochondria. The latter are also seen breaking. The albuminous yolk

Plate I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Figs. 15–19
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50. Nath, Vishwa.—Studies in the shape of Golgi apparatus. Egg follicle of Culex. Ztschr. F. Zellf. Mikr. Anat. 1929.

51. Nath, Vishwa.—Studies on the shape of Golgi apparatus II observation on the fresh egg of the Indian earthworm Pheritima posthuma. Q.J.M.S. 73. 1930.

52. Nath, Vishwa.—On the present position of the Golgi apparatus and mitochondria. Biol. Review. Vol. 2, No. 1. Cambridge.

53. Nath, Vishwa, and Hussain.—Oogenesis of Scolopendra. Q.J.M.S. 72, 403. 1928.

54. Nath, Vishwa, and Mehta.—Oogenesis of fire-fly Luciola. Q.J.M.S. 1929.

55. Nath, Vishwa, and Mohan.—Oogenesis of cockroach. Jour. Morph. and Physio. Vol. 48. No. 1. 1929.

56. Pappenheimer, A. M.—The Golgi apparatus. Anat. Sci., No. 11. 107—48, 1916.

57. Parat, M.—Vacuome et appareil de Golgi. C. R. de L'associ. des anat. pp. 306—11.

58. Parat, M.—1926 a Sur la constitution de L' appareil de Golgi et de l'idiosome; vrais et faux dictyosomes. C. R. de L' Acad. des Scit., CLXXXII. pp. 808-09.

59. Parat, M.—1926 b Vacuome et appareil de Golgi. C. R. de L'assoc. des anat.

60 Parat, M.—1927 b Evolution de vacuome au cours ou l'orogeneses el l'ontogenese son importance physiologique ches l'adulte. C. R. de L'Assoc. des anat. 197—202.

61. Parat, M.—1927 a les colarations Vitales des cellules animales d apres von Mollendraff publications a chahine. Paris. Fasc. 3p. 1—37.

62. Parat, M.—1928 a Sur los r apports du chondriomes et du vacuome dans la zone de Golgi constitution de cetu zone. C. R. de L' Assoc. des Anat.

63. Parat, M. and Bhattacharya, D. R.—Les constituents cytoplasmiques de la cellule genitale femelle L'ovocyte de Ciona intestinalis. C. R. de la Soc. de Bio. T. XCIV. pp. 444—46. 1926.

64. Parat, M. and Painleve, J.—1925 c Mise en evidence

32. Gresson, R. A. R.—Yolk formation in *Periplaneta orientalis*. Q. J. M. S., Vol. 74, Pt. II, Jan. 1931.

33. Harvey, L. A.—The history of the cytoplasmic inclusions of the egg of Ciona intestinalis. Proc. Roy. Soci., B. 101. 136. 1927.

34. Harvey, L. A.—The oogenesis of Carcinus-maenus-Penn. With special reference to yolk formation. Trans. Roy. Soc., Edin. Vol. 56, 1929.

35. Hibbard, H.—Cytoplasmic constituents in the developing egg of Discoglosus pictus. Jour. Morph., Vol. 45, 1928.

36. Hogben, L. T.—Studies on synapsis 1 and 2. Proc. Roy. Soc., B. 91. 1920, pp. 268 and 305.

37. King, S. D.—Oogenesis in Lithobius forficatus. So. Proc. Roy. Dubl. Soc. (N. S.), 18, 29. 1924.

38. King, S. D.—Oogenesis in Oniscus asellus. Proc. Roy. Soc., B. 100, 1, 1926.

39. Loyez, M.—Recherches sur le developpment ovarien des ecuts meroblastiquesa vitellus nutritif abondant. Arch. d'Anat. Micros., 8. 1905 and 1906.

40. Ludford, R. J.—Contribution to the study of oogenesis of Patella. Jour. Roy. Micr. Soci., 41, 1. 1921.

41. Ludford, R. J.—Further modifications of the osmic acid methods in the cytological technique. Ibid., Jan. 1926.

42. Ludford, R. J. and Gatenby, J. B.—*Dictyokinesis in Germ cells*. Proc. Roy. Soci., B. Vol. 92, 1921.

43. Lyon, E. P.—Results of centrifuging egg. Archi. Fur. Entwi. Mech. XXIII. Berlin. 1907.

44. Morse, M.—The nuclear components of the sex cells of four species of cockroaches. Arch. Zellf. Band 3, 1919.

45. Munson, J. P.—A comparative study of structure and origin of yolk nucleus. Ibid., Band 8, 1912.

46. Nath, Vishwa—Cell inclusions in the oogenesis of scorpion. Proc. Roy. Soci., Lond. B. 99, 44. 1925.

47. Nath, Vishwa.—*Oogenesis of Lithobius forficatus*. Proc. Camb. Phil. Soc., Biol. Sci. 1926.

48. Nath, Vishwa.—The Golgi origin of fatty yolk in the light of Parat's work. Nature, Nov. 1927.

49. Nath, Vishwa.—Studies in the origin of yolk oogenesis of spider Crossoperiza Lyons Blackwell. Q. J. M. S., 72, 1928, pp. 228—300.

16. *Dutta and Asana.*—On behaviour of the Golgi apparatus in the oogenesis of Calotes versicolor (Boulenger). Zoology Department, University of Allahabad.

17. D'Hollander, F. G.—Arch. D'Anat. Micr. 1904.

18. Das, R. S.—Cytoplasmic inclusions in the oogenesis of birds. (In Press.)

19. Fano, C. Da.—Methods for demonstration Golgi's internal apparatus. Jour. of Physio. 53, XCLH. 1920 B.

20. Gatenby, J. B. and Woodger, J. H.—On the relationship between the formation of yolk and mitochondria, Golgi apparatus during oogenesis. Jour. Roy. Soci. Micros. Part II, 1920.

21. Gatenby, J. B.—On the relationship between the formation of yolk, mitochondria, and Golgi bodies during oogenesis. Jour. Roy. Micr. Soci., 151-121-136.

22. Gatenby, J. B.—Cytoplasmic inclusions in the germ cell. The Gametogenesis of Saccocirrus. Q. J. M. S., 66. 1—48.

23. Gatenby, J. B. and Cowdry, E. V.—Microtomists' Vade-Mecum.

24. Gatenby, J. B.—Study of Golgi apparatus and vacuolar system of Caria, Helix and Abraxas by intra-vital methods. Proc. Roy. Soc., B. Vol. 104. 1929.

25. Gatenby, J. B.—The cytoplasmic inclusions of the germ cells. Part II, Helix aspara. Q. J. M. S., LXII, 1927.

26. Gatenby, J. B.—The cytoplasmic inclusions of the germ cells. Part V. The gametogenesis and early development of Limnaea stagnalis with special reference to the Golgi apparatus and mitochondria. Q. J. M. S., LXIV, 1919.

27. Gatenby, J. B.—Oogenesis of Lumbricus. Nature, 118. 840. 1926.

28. Gatenby, J. B.—Cell nomenclature. Jour. Roy. Micro. Soc., Vol. L. 20—29. 1930.

29. Gatenby, J. B. and Nath, V.—The oogenesis of certain invertebrata with special reference to Lumbricus. Q. J. M. S. Vol. 70.-Part III.

30. Glaser, R. W.—On the isolation, cultivation and classification of the so-called intra-cellular symbionts or "Rikettsia" of Periplaneta americana. Jour. Exptl. Med., Vol. 51. No. 1. 1930.

31. Gresson, R. A. R.—Yolk formation in certain saw-flies of the family tenthredinidæ. Q. J. M. S., Vol. 73. Pt. II, 1929.

32. Gresson, R. A. R.—Yolk formation in Periplaneta orientalis. Q. J. M. S., Vol. 74, Pt. II, Jan. 1931.

33. *Harvey, L. A.—The history of the cytoplasmic inclusions of* the egg of Ciona intestinalis. Proc. Roy. Soci., B. 101. 136. 1927.

34. Harvey, L. A.—The oogenesis of Carcinus-maenus-Penn. With special reference to yolk formation. Trans. Roy. Soc., Edin. Vol. 56, 1929.

35. Hibbard, H.—Cytoplasmic constituents in the developing egg of Discoglosus pictus. Jour. Morph., Vol. 45, 1928.

36. Hogben, L. T.—Studies on synapsis 1 and 2. Proc. Roy. Soc., B. 91. 1920, pp. 268 and 305.

37. King, S. D.—Oogenesis in Lithobius forficatus. Sc. Proc. Roy. Dubl. Soc. (N. S.), 18, 29. 1924.

38. King, S. D.—*Oogenesis in Oniscus asellus. Proc. Roy. Soc., B.* 100, 1, 1926.

39. Loyez, M.—Recherches sur le developpment ovarien des oeufs meroblastiquesa vitellus nutritif abondant. Arch. d'Anat. Micros., 8. 1905 and 1906.

40. Ludford, R. J.—Contribution to the study of oogenesis of Patella. Jour. Roy. Micr. Soci., 41, 1. 1921.

41. Ludford, R. J.—Further modifications of the osmic acid methods *in the cytological technique. Ibid., Jan.* 1926.

42. Ludford, R. J. and Gatenby, J. B.—Dictyokinesis in Germ cells. Proc. Roy. Soci., B. Vol. 92, 1921.

43. Lyon, E. P.—Results of centrifuging egg. Archi. Fur. Entwi. Mech. XXIII. Berlin. 1907.

44. Morse, M.—The nuclear components of the sex cells of four species of cockroaches. Arch. Zellf. Band 3, 1919.

45. Munson, J. P.—A comparative study of structure and origin of yolk nucleus. Ibid., Band 8, 1912.

46. Nath, Vishwa—Cell inclusions in the oogenesis of scorpion. Proc. Roy. Soci., Lond. B. 99, 44. 1925.

47. Nath, Vishwa.—Oogenesis of Lithobius forficatus. Proc. Camb. Phil. Soc., Biol. Sci. 1926.

48. Nath, Vishwa.—The Golgi origin of fatty yolk in the light of Parat's work. Nature, Nov. 1927.

49. *Nath, Vishwa.—Studies in the origin of yolk* oogenesis of spider Crossoperiza Lyons Blackwell. Q. J. M. S., 72, 1928. pp. 228—300.

50. Nath, Vishwa.—Studies in the shape of Golgi apparatus. Egg follicle of Culex. Ztschr. F. Zellf. Mikr. Anat. 1929.

51. Nath, Vishwa.—Studies on the shape of Golgi apparatus II observation on the fresh egg of the Indian earthworm Pheritima posthuma. Q.J.M.S. 73. 1930.

52. Nath, Vishwa – On the present position of the Golgi apparatus and mitochondria. Biol. Review, Vol. 2, No. 1. Cambridge.

53. Nath, Vishwa, and Hussain.—Oogenesis of Scolopendra. Q.J.M.S. 72, 403. 1928.

54. Nath, Vishwa, and Mehta.—Oogenesis of fire-fly Luciola. Q.J.M.S. 1929.

55. Nath, Vishwa, and Mohan.—Oogenesis of cockroach. Jour. Morph. and Physio. Vol. 48, No. 1, 1929.

56. Pappenheimer, A. M.—The Golgi apparatus. Anat. Sci., No. 11. 107—48, 1916.

57. Parat, M.—Vacuome et appareil de Golgi. C. R. de L'associ. des anat. pp. 306—11.

58. Parat, M.—1926 a Sur la constitution de L' appareil de Golgi et de l'idiosome; vrais et faux dictyosomes. C. R. de L' Acad. des Scit., CLXXXII. pp. 808-09.

59. Parat, M.—1926 b Vacuome et appareil de Golgi. C. R. de L'assoc. des anat.

60. Parat, M.—1927 b Evolution de vacuome au cours ou l'ovogeneses et l'ontogenese son importance physiologique ches l'adulte. C. R. de L'Assoc. des anat. 197—202.

61. Parat, M.—1927 a les colarations Vitales des cellules animales d apres von Mollendraff publications a chabine. Paris. Fasc. 3p. 1—37.

62. Parat, M.—1928 a Sur los r apports du chondriomes et du vacuome dans la zone de Golgi constitution de cetu zone. C. R. de L' Assoc. des Anat.

63. Parat, M. and Bhattacharya, D. R.—Les constituents cytoplasmiques de la cellule genitale femelle L'ovocyte de Ciona intestinalis. C. R. de la Soc. de Bio. T. XCIV. pp. 444—46. 1926.

64. Parat, M. and Painleve, J.—1925 c Mise en evidence de vacuome (appareil reticulaire de Golgi), etc., de L' appareil de Golgi classique. C. R. de L' Acad. des Sciences. T. CLXXX, pp. 1134—1136.

65. Rai, H. S.—On the origin of yolk in the egg of Oisteren cucullate. Jour. Rou. Micro. Soci, 3 Vol. L. Pt II. 1930.

66. Sarga, U. S.—Cytoplasmic inclusions in the oogenesis of P. posthuma. Alld. Univ. Studies, Vol. 4.

67. Subba Rau and Brambell, F. W. R.—Staining methods for the demonstrations of the Golgi apparatus in fresh vertebrates and invertebrates material. Jour. Micro. Soci. 1925, p. 438.

68. Subba Rau and Gatenby, J. B.—Notes on the distribution, morphology, and cytology of the organ of Bidder. Jour. Roy. Micro. Soci. 1922.

69. Subba Rau and Ludford, R. J.—Variations in the form of the Golgi bodies during the development of neuron. Q. J. M. S., Vol. 69, Pt. 3. 1925.

70. Waldeyer—Eierstock und Ei Leipzig. 1870.

71. Naryan Rao, C. R.—On the structure of the ovary and the ovarian ovum of Lorisiydek kerianus, Cabr. Q. J. M. S., Vol. 71, 1928.

THE GOLGI BODIES IN THE ERYTHROCYTES OF FISHES

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INTRODUCTION

The occurrence of Golgi bodies in somatic and germ cells has become an established fact owing to the researches of cytologists during the last thirty years. In 1925, (1) the senior author (D. R. B.) and Brambell were able to demonstrate the occurrence of Golgi bodies in the erythrocytes of reptiles and birds. No further work of importance seems to have been done in this direction since then except by Dawson (5) who has studied the reaction of Amphibian erythrocytes to vital dyes. For throwing further light on the subject, a number of fishes of different species were examined by us and all possible precautionary measures were taken in order to avoid artifacts. The best results were obtained by Ludford's modification of Mann-Kopsch technique (6). Da Fano's silver method (5) was tried as well but due to poor fixation the results were not very satisfactory. In both cases it was noticed that Golgi bodies were present as discrete elements scattered in the cytoplasm of the erythrocytes of Ophiocephalus punctatus, which was studied in particular (Figs. 1—9). The Golgi bodies are spherical in shape and occasionally lie in a juxta-nuclear position (Figs. 1, 2, 4 and 9). In some red blood-cells they appear to be plastered, as it were, around the nuclear membrane. In all cases the Golgi bodies are very minute and granular in size and are visible only under very high magnifications.

DISCUSSION

Since the discovery of Golgi apparatus by the Italian neurologist Golgi in 1898, this cell component has been described in nearly all categories of cells. Cowdry in 1914 (3) demonstrated the occurrence of mitochondria by vital staining methods but felt sceptic about the presence of Golgi bodies in erythrocytes. It was with a view to fill up this gap that Bhattacharya and Brambell described the presence of Golgi bodies in reptiles and birds and suggested that " In view of the phylogenetic relationships existing between the Sauropsida and the other classes it seems probable that Golgi bodies will be found to occur not only in the nucleated red-blood corpuscles of the fishes and amphibians, but also in the non-nucleated red-blood corpuscles of the mammals, at least in some stages of their development." The present work is intended to supplement the knowledge gained by the works of the above-mentioned authors. The fishes differ from the Sauropsida in that the Golgi bodies lie generally scattered throughout the cytoplasm in a diffused condition and in much larger numbers. The ground cytoplasm of the red blood-cell appears to be smooth and homogeneous as is also the case in reptiles and birds. Regarding the structure of the Golgi bodies little can be said because of their extremely minute size. They appear to be spherical but the chromophobic core can hardly be distinguished from the chromophilic cortex as is the case in many somatic and germ cells. That they are not artifacts is demonstrated by the fact that their presence was noted both by osmic and silver nitrate techniques. The slides were toned, washed and bleached, as the case may be, for varying lengths of time. The result in all cases was practically the same—thus proving without doubt that Golgi elements in granular form are present in the erythrocytes of fishes either in a diffused condition throughout the cytoplasm or in a juxta-nuclear position as discrete elements or plastered around the nucleus in a perinuclear position.

ADDENDUM

Since going to the press, a copy of a paper by A. B. Dawson, on the reaction of erythrocytes to vital dyes has reached our hands. The author concerns himself mostly to an examination of erythrocytes by vital staining methods and concludes that in vertebrate erythrocyte, the primary granules, the secondary granules and patterns of reticulum as revealed by vital dyes, are to be regarded as three separate entities which are not genetically related. Dornesco and Steopoe (8 and 9) carried on vital staining experiments on certain fishes and have tried to prove that Golgi elements and Vacuome are homologous structures in the erythrocytes of fishes. Our position remains unaltered, for we hold with Gatenby, Bowen and others that what is revealed with the aid of osmic acid and silver salts by the classical methods are Golgi bodies and that neutral red may be specific for what are called vacuome, but not for what are known as Golgi elements. The question of homology, therefore, does not seem to arise.

REFERENCES

1. Bhattacharya, D. R., Brambell, and F. W. Rogers.—The Golgi body in the erythrocytes of the Sauropsida. Q. J. M. S. Vol. 69, Pt II. 1925.
2. Cowdry, E. V.—General Cytology.
3. Ibid.—Special Cytology, Vol. I, p. 314.
4. Ibid.—The vital staining of mitochondria with Janus green and diethyl-safranin in human blood cells.
5. Dawson, A. B.—A Further Study of the Reaction of the Amphibian Erythrocyte to Vital Dyes, Osmic Acid and Silver Salts, with Special Reference to Basophilia and Reticulum. Anat. Rec., 42, 281. 1929.
6. Bolle's, Lee.—Microtomists, Vade mecum. 1928.
7. Ludford, J. R.—Further modifications of the Osmic acid methods in Cytological technique. Jour. Roy. Micr. Soc., June 1926.
8. Dornesco, G. Th., et J. Steopoe.—L'appareil de Golgi des globules rouges des Téléostéens marins. Compt. rend. Soc. Biol. 105, 288. 1930 a.

9. Dornesco, G. Th., et J. Steopoe.—L'appareil de Golgi des hématies dés Selaciens. Compt. rend. Soc. Biol., 105, 446. 1930 b.

DESCRIPTION OF PLATE 1.

The figures were drawn with the help of Camera lucida, Leitz, under oil immersion lens.

Figs. 1—7—Ludford's Osmic Technique.

Fig. 1.—Side view of a red blood cell of Ophiocephalus punctatus showing a few Golgi bodies (GB) situated close to the nucleus.

Fig. 2.—Same as figure 1. Surface view showing the perinuclear arrangement of Golgi bodies.

Figs. 3 & 4.—Surface view of erythrocytes. In the latter the Golgi bodies are arranged in a juxta-nuclear position.

Fig. 5.—Side view of red blood-cell showing Golgi bodies plastered round the nuclear wall.

Fig. 6.—Surface view of Figure 5.

Fig. 7.—Surface view of a red blood-cell showing Golgi bodies scattered throughout the cytoplasm.

Figs. 8 & 9.—Da Fano's silver method.

Surface view of two erythrocytes showing the Golgi granules situated close to the nucleus.

PLATE I